EFFECT OF BREWERY WASTE ON NUTRIENT DIGESTIBILITY OF PADDY STRAW BASED DIET IN LACTATING COWS

By

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DECLARATION

I hereby declare that the thesis entitled **"EFFECT OF BREWERY WASTE ON NUTRIENT DIGESTIBILITY OF PADDY STRAW BASED DIET IN LACTATING COWS"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Certified that this thesis entitled **"EFFECT OF BREWERY WASTE ON NUTRIENT DIGESTIBILITY OF PADDY STRAW BASED DIET IN LACTATING COWS"** is a record of research work done independently by **Dr. S. Senthilkumar** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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Dedicated to

Tomorrow's Thought Leaders of

Our Beloved Motherland

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1. INTRODUCTION

Cereal crop residues form the staple feed for ruminant livestock in India. Among the various cereal crop residues, paddy straw (*Oryza sativa*) is the main roughage source for majority of cattle and buffaloes reared in Indian small holdings. However, the nutrient digestibility of straw is very poor. Low degradability of paddy straw is due to its high lignin content and shortage of essential nutrients for rumen microbes. This has led to search for appropriate treatments and supplements to improve its nutritive value. There has been substantial attention to treatments, but less to supplementation strategies. The first limitation of straw is the imbalance of nutrients both for rumen microbes and for the host animal. Therefore, supplements that are nutritionally synergistic with paddy straw are of great interest to improve voluntary intake, rate and extent of fermentation of its cell walls and absorption of nutrients. A wide range of agro-industrial byproducts are available in large quantities which have considerable nutritional potential. Among them, brewery waste (Brewer's grains) is a typical example of such unrealized potential. Brewery waste is a by product of ethanol industry which uses cereal grains as feed stock. When grain is fermented to produce ethanol, primarily the starch is utilized, leaving behind a protein rich residue that can be used in livestock diets.

India ranks fifth in the world in ethanol production (Economic Times, 2006) and produces ethanol from about 278 distilleries, majority of which use agricultural crops as feed stock. One ton of grain on fermentation yields 430 kg of distiller's grain (Rendell, 2004). As the ethanol industry grows, greater quantities of distiller's grain will become available for use as animal feed at potentially reasonable cost. Distiller's grain has a moderate content of protein and high level of crude fibre which make it an attractive ingredient to be used in ruminant feed (Rasco *et al*., 1989).

In spite of its abundant availability, the important factors which limits to utility as animal feed is their short shelf life (3 to 7 days) due to high moisture and transportation difficulty (Rendell, 2004).

Knowledge on degradability and rumen fermentation pattern of brewery waste incorporated with paddy straw would throw more light on its usefulness and would help to formulate complete feed for dairy cattle.

Hence, an attempt was made in the present study to evaluate the efficiency of utilization of brewery waste incorporated paddy straw based diet in lactating cows. The *in vitro* rumen fermentation pattern of brewery waste incorporated paddy straw based feed for dairy cattle was also studied. The objectives set forth in this study were:

- To study the dry matter and fibre degradability pattern of brewery waste incorporated feed with paddy straw by *in vitro* method using Rumen Simulation Technique (RUSITEC).

- To evaluate the effect of brewery waste on dry matter digestibility of paddy straw based ration by *in vivo* method.

- To assess the lactational performance of crossbred cows maintained on brewery waste based concentrate feed with paddy straw as the sole roughage.

- To assess the cost of production by feeding brewery waste in the diet for lactating crossbred cows.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 CHEMICAL COMPOSITION OF PADDY STRAW

2.1.1 Proximate Composition

The dry matter (DM) content of paddy straw was 88 to 90 per cent as reported by Ram *et al.* (1985), Reddy and Reddy (1989), Keir *et al.* (1997), Ally (2003), Joseph (2005), Augustine (2008) and Nader and Robinson (2008). However, lower value of 82.4 per cent was reported by Nguyen and Uden (2001) and 87.7 per cent by Singh *et al.* (2002).

The crude protein (CP) content of paddy straw was 3 to 4 per cent as reported by Ramanathan (1979), Ram *et al.* (1985), Borah *et al.* (1988), Srinivas and Gupta (1991), Keir *et al.* (1997), Dohnani *et al.* (2001), Garg *et al.* (2002) and Singh *et al.* (2002). Higher CP value of 5 per cent was reported by Krishnamoorthy *et al.* (1995), Reddy (1997), Mishra *et al.* (2000) and Ally (2003). Still higher CP values of 6.1 and 5.6 per cent were reported by Nguyen and Uden (2001) and Nader and Robinson (2008), respectively.

The ether extract (EE) content of paddy straw was from 1 to 2 per cent as reported by Ram *et al.* (1985), Borah *et al.* (1988), Reddy and Reddy (1989), Srinivas and Singh (1998), Garg *et al.* (2002), Joseph (2005) and Augustine (2008). Lower EE value of 0.78 per cent was reported by Krishnamoorthy *et al.* (1995) and higher value of 3.48 per cent was reported by Ally (2003).

The crude fibre (CF) content of paddy straw was ranged from 26 to 33 per cent as reported by Ramanathan (1979), Ram *et al.* (1985), Ally (2003), Joseph (2005) and Augustine (2008), while, Borah *et al.* (1988) and Srinivas and Gupta (1991) reported CF value of 38 and 39.4 per cent, respectively. Lower CF value of 23.17 per cent was reported by Reddy and Reddy (1989).

The total ash content of paddy straw was 14 to 17 per cent as reported by Ram *et al.* (1985), Reddy and Reddy (1989), Srinivas and Gupta (1991), Krishnamoorthy *et al.* (1995), Mishra *et al.* (2000), Garg *et al.* (2002), Joseph (2005), Augustine (2008) and Nader and Robinson (2008). Lower total ash value of 7.3 per cent was reported by Ramanathan (1979) and higher value of 18.35 per cent was reported by Singh *et al.* (2002).

2.1.2 Fibre Fractions

The neutral detergent fibre (NDF) content of paddy straw was 70 to 80 per cent as reported by Rai and Mudgal (1987), Rai *et al.* (1989), Reddy *et al.* (1993), Bae *et al.* (1997), Reddy (1997), Mishra *et al.* (2000), Dohnani *et al.* (2001), Gangwar and Sharma (2001), Nguyen and Uden (2001), Singh *et al.* (2001), Sohane and Singh (2001), Singh *et al.* (2002), Ally (2003) and Joseph (2005). Higher NDF value of 84.2 per cent was reported by Ramanathan (1979) and lower value of 66.6 per cent was reported by Cann *et al.* (1991) and 67.7 per cent by Garg *et al.* (2002).

The acid detergent fibre (ADF) content of paddy straw was 45 to 52 per cent as reported by Rangnekar *et al.* (1982), Rai and Mudgal (1987), Reddy *et al.* (1993), Bae *et al.* (1997), Reddy (1997), Mishra *et al.* (2000), Dohnani *et al.* (2001), Singh *et al.* (2001), Singh *et al.* (2002), Garg *et al.* (2002), Ally (2003), Joseph (2005) and Nader and Robinson (2008). Higher ADF value of 53.8 per cent was reported by Gangwar and Sharma (2001) and lower value of 39.07 per cent was reported by Adeloye (2001).

The hemicellulose content of paddy straw was 20 to 30 per cent as reported by Rai and Mudgal (1987), Cann *et al.* (1991), Reddy *et al.* (1993), Bae *et al.* (1997), Reddy (1997), Srinivas and Singh (1998), Mishra *et al.* (2000), Dohnani *et al.* (2001), Nguyen and Uden (2001), Singh *et al.* (2001), Sohane and Singh (2001), Singh *et al.* (2002) and Nader and Robinson (2008). Higher hemicellulose value of 41.64 per cent was reported by Adeloye (2001) and lower value of 16.6 per cent was reported by Gangwar and Sharma (2001).

The cellulose content of paddy straw was 30 to 40 per cent as reported by Rangnekar *et al.* (1982), Rai and Mudgal (1987), Rai *et al.* (1989), Reddy *et al.*

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The lignin content of paddy straw was 5 to 10 per cent as reported by Rai *et al.* (1989), Cann *et al.* (1991), Reddy *et al.* (1993), Bae *et al.* (1997), Reddy (1997), Srinivas and Singh (1998), Mishra *et al.* (2000), Adeloye (2001), Dohnani *et al.* (2001), Nguyen and Uden (2001), Singh *et al.* (2001), Sohane and Singh (2001), Singh *et al.* (2002), Ally (2003) and Nader and Robinson (2008). Higher lignin value of 11.2 per cent was reported by Rangnekar *et al.* (1982), while lower value of 2.70 per cent was reported by Garg *et al.* (2002).

The acid insoluble ash (AIA) content of paddy straw was 7 to 8 per cent as reported by Singh *et al.* (1995) and Garg *et al.* (2002). Higher AIA value of 12.61 per cent was reported by Sohane and Singh (2000).

Chaudhry (1998) reported that cereal straws were high in lignin and silica, which limited their digestibility.

2.1.3 Minerals

The reported calcium, phosphorus and magnesium content of paddy straw did not vary much but the micro minerals such as copper, zinc, iron and manganese varied widely. The calcium content of paddy straw was 0.23 to 0.50 per cent as reported by Ranjhan (1993), Singh *et al.* (1995), Banerjee (1998), Ramana *et al.* (2000), Ally (2003), Joseph (2005) and Nader and Robinson (2008). The phosphorus content of paddy straw was 0.08 to 0.18 per cent as reported by Ranjhan (1993), Banerjee (1998), Ramana *et al.* (2000), Ally (2003) and Nader and Robinson (2008). The magnesium content of paddy straw was on an average of 0.17 per cent as reported by Banerjee (1998), Ramana *et al.* (2000) and Nader and Robinson (2008). The potassium content of paddy straw was 1.32 per cent as reported by Ensminger *et al.* (1990). The copper content of paddy straw was 6 to 10 ppm as reported by Ranjhan (1993), Banerjee (1998), Ramana *et al.* (2000) and Nader and Robinson (2008). The zinc content of paddy straw was on an average of 5 ppm as reported by Ramana *et al.* (2000). The iron content of paddy straw was 190 to 250 ppm as reported by Ranjhan (1993), Ramana *et al.* (2000) and Nader and Robinson (2008). The manganese content of paddy straw was 55 to 110 ppm as reported by Ranjhan (1993) and Banerjee (1998).

2.1.4 Nutritive Value of Paddy Straw

Ranjhan (1993) reported that paddy straw contained 0.3 per cent digestible crude protein (DCP) and 40.5 to 51.8 per cent total digestible nutrients (TDN). Similar values for DCP (0.2 to 0.5 per cent) and TDN (40.5 to 45.9 per cent) were reported by Banerjee (1998).

The low nutritive value of paddy straw was attributed to the presence of indigestible lignin, silica and the manner in which it was bound to digestible cellulose and hemicellulose. This depressed the voluntary feed intake and digestibility of straw (Wanapat, 1984 and Doyle *et al*., 1986). Doyle (1989) reported the supplementation of nutrients that are limiting in paddy straw as the most practical means of improving the nutrient utilization from paddy straw. Chowdhury (2001) reported that the low content of readily fermentable energy, nitrogen, minerals and vitamins as the nutritional constraints of paddy straw.

2.2 BREWER’S GRAIN AND DISTILLER’S GRAIN

2.2.1 Chemical Composition of Brewer’s Grain

2.2.1.1 Proximate Composition

The DM content of wet brewer’s grain was 25 to 30 per cent as reported by Murdock *et al*. (1981), Davis *et al*. (1983) and Dong and Ogle (2003). Lower DM value of 18.6 per cent was reported by Rogers *et al*. (1986), while a higher value of 33.6 per cent was reported by Dhiman *et al*. (2003).

The CP content of brewer’s grain was 23 to 28 per cent as reported by Murdock *et al*. (1981), Davis *et al*. (1983), Rogers *et al*. (1986), Devendra

(1992), Belibasakis and Tsirgogianni (1996), Batajoo and Shaver (1998), Dong and Ogle (2003) and Dhiman *et al*. (2003). Higher CP value of 30.1 per cent was reported by Crickenberger and Johnson (1982) and lower value of 18.5 per cent was reported by Hayashi *et al*. (2005).

The CF content of brewer's grain was 15 to 20 per cent as reported by Onwudike (1986), Devendra (1992), Belibasakis and Tsirgogianni (1996) and Dong and Ogle (2003). The ether extract content of brewer's grain was 7 to 10 per cent as reported by Batajoo and Shaver (1998) and Dong and Ogle (2003). The total ash content of brewer's grain was 3 to 5 per cent as reported by Dong and Ogle (2003). The NFE content of brewer's grain was 40 to 50 per cent as reported by Dong and Ogle (2003).

2.2.1.2 Fibre Fractions

The NDF content of brewer's grain was 50 to 55 per cent as reported by Murdock *et al*. (1981), Depeters *et al*. (1997), Batajoo and Shaver (1998), Dong and Ogle (2003). Higher NDF value of 70 per cent was reported by Dhiman *et al*. (2003) and lower value of 40 to 50 per cent was reported by Belibasakis and Tsirgogianni (1996) and Hayashi *et al*. (2005).

The ADF content of brewer's grain was on an average of 20 per cent as reported by Murdock *et al*. (1981), Davis *et al*. (1983), Rogers *et al*. (1986) and Depeters *et al*. (1997). Higher ADF value of 27.7 per cent was reported by Dhiman *et al*. (2003), while a lower value of 17.5 per cent was reported by Dong and Ogle (2003).

The lignin content of brewer's grain was on an average of five per cent as reported by Murdock *et al*. (1981) and Depeters *et al*. (1997).

2.2.1.3 Minerals

The calcium content of brewer's grain was around 0.3 per cent as reported by Crampton and Harris (1969), Titus and Fritz (1971), Sullivan *et al.* (1978), Murdock *et al.* (1981), Crickenberger and Johnson (1982), Scott *et al.* (1982) and

Dong and Ogle (2003). Lower calcium value of 0.16 per cent was reported by Gohl (1981).

The phosphorus content of brewer's grain was between 0.5 and 0.8 per cent as reported by Crampton and Harris (1969), Titus and Fritz (1971), Sullivan *et al.* (1978), Gohl (1981), Murdock *et al.* (1981), Crickenberger and Johnson (1982), Scott *et al.* (1982), Dong and Ogle (2003) and Dhiman *et al.* (2003).

The magnesium content of brewer's grain was 0.26 per cent as reported by Dhiman *et al.* (2003). Higher magnesium value of 0.54 per cent was reported by Dong and Ogle (2003).

The sodium content of brewer's grain was around 0.26 per cent as reported by Crampton and Harris (1969), Titus and Fritz (1971) and Dong and Ogle (2003). Lower sodium value of 0.02 to 0.04 per cent was reported by Scott *et al.* (1982) and Dhiman *et al.* (2003).

The potassium content of brewer's grain was 0.46 per cent as reported by Dhiman *et al.* (2003). Lower potassium value of 0.08 to 0.13 per cent was reported by Crampton and Harris (1969), Titus and Fritz (1971) and Scott *et al.* (1982).

Dhiman *et al.* (2003) reported that the iron content of brewer's grain was between 100 and 125 ppm. Higher iron value of 330 ppm was reported by Dong and Ogle (2003).

Dhiman *et al.* (2003) reported 80 to 85 ppm of zinc and 15 ppm of copper in brewer's grain, while Dong and Ogle (2003) reported lower copper value of 7.7 ppm

The manganese content of brewer's grain was between 30 and 50 ppm as reported by Dong and Ogle (2003) and Dhiman *et al.* (2003).

Brewer's grain contained 0.32 ppm of selenium (Murdock *et al.*, 1981) and 1.5 to 1.8 ppm of molybdenum (Dhiman *et al.*, 2003).

2.2.1.4 Vitamins and Amino Acids

Mariani (1953) and Huige (1994) reported that brewer's grain contained 0.1 ppm of biotin, 1800 ppm of choline, 0.2 ppm of folic acid, 44 ppm of niacin, 8.5 ppm of pantothenic acid, 1.5 ppm of riboflavin, 0.7 ppm of thiamine and 0.7 ppm of pyridoxine.

The amino acids present in brewer's grain include protein bound amino acids such as leucine, valine, alanine, serine, glycine, glutamic acid and aspartic acid in larger amounts, and tyrosine, proline, threonine, arginine and lysine in smaller amounts. The presence of cystine, histidine, isoleucine, methionine, phenylalanine and tryptophan also was reported (Mariani, 1953 and Huige, 1994).

Brewer's spent grain can also used as substrate for the production of cellulase complex enzymes by *Trichoderma resei* (Sim and Oh, 1990 and Bartolome *et al.*, 2002). The germination step in beer making promotes the synthesis and activation of enzymes such as amylases, proteases and β-glucanases in the aleurone and starchy endosperm (Bartolome *et al.*, 2002).

2.2.2 Chemical Composition of Distiller's Grain

2.2.2.1 Proximate Composition

The distiller's grain contained 30 to 35 per cent DM as reported by Weiss *et al.* (1989), Mustafa *et al.* (2000b), Al-Suwaiegh *et al.* (2002), Rendell (2004) and Birkelo *et al.* (2004).

The CP content of distiller's grain was around 30 per cent as reported by Weiss *et al.* (1989), Carroll *et al.* (1997), Al-Suwaiegh *et al.* (2002), Rendell (2004) and Charles *et al.* (2005). Lower CP value of 20.1 per cent was reported by Mustafa *et al.* (2000b), while a higher value of 39.5 per cent was reported by Birkelo *et al.* (2004).

The CF content of distiller's grain was 15 per cent as reported by Chenost and Mayer (2001). Lower CF value of 9 per cent was reported by Spiehs *et al.* (2002).

Distiller's grain was reported to have 5 to 10 per cent of ether extract (Carroll *et al*., 1997; Mustafa *et al*., 2000b; Rendell, 2004 and Birkelo *et al*., 2004). Higher EE value of 14.5 per cent and a lower value of 3.78 per cent were reported by Al-Suwaiegh *et al.* (2002) and Charles *et al.* (2005), respectively.

The total ash content of distiller's grain was around 5 per cent as reported by Mustafa *et al.* (2000b), Birkelo *et al.* (2004) and Charles *et al.* (2005).

2.2.2.2 Fibre Fractions

The NDF content of distiller's grain was 45 to 60 per cent as reported by Weiss *et al.* (1989), Schingoethe *et al.* (1999), Al-Suwaiegh *et al.* (2002) and Birkelo *et al.* (2004). Lower NDF value of 34.17 per cent was reported by Charles *et al.* (2005).

The ADF content of distiller's grain was 20 to 30 per cent as reported by Weiss *et al.* (1989), Carroll *et al.* (1997), Schingoethe *et al.* (1999), Al-Suwaiegh *et al.* (2002), Birkelo *et al.* (2004) and Charles *et al.* (2005).

2.2.2.3 Minerals

The calcium content of distiller's grain was 0.1 to 0.3 per cent as reported by Shelford and Tait (1986), Weiss *et al.* (1989), Huang *et al.* (1999), Schingoethe *et al.* (1999), Dale and Batal (2003) and Kleinschmit *et al.* (2006). However, Fastinger and Mahan (2006) reported a higher calcium content of 0.7 per cent in distiller's grain.

Distiller's grain had 0.5 to 0.9 per cent of phosphorus as reported by Shelford and Tait (1986), Weiss *et al.* (1989), Schingoethe *et al.* (1999), Spiehs *et al.* (2002), Dale and Batal (2003), Shurson *et al.* (2004) and Kleinschmit *et al.*

(2006). Lower phosphorus value of 0.26 per cent was reported by Huang *et al.* (1999).

Shelford and Tait (1986), Weiss *et al.* (1989), Huang *et al.* (1999), Schingoethe *et al.* (1999), Spiehs *et al.* (2002), Dale and Batal (2003), Shurson *et al.* (2004) and Kleinschmit *et al.* (2006) reported the magnesium content of distiller's grain as 0.2 to 0.4 per cent.

The sodium content of distiller's grain was 0.3 to 0.5 per cent as reported by Shelford and Tait (1986), Huang *et al.* (1999) and Spiehs *et al.* (2002), while a lower sodium content of 0.13 per cent was reported by Lumpkins *et al.* (2004).

The potassium content of distiller's grain was reported as 0.16 per cent (Shelford and Tait, 1986) and as 0.6 to 0.9 per cent (Spiehs *et al.*, 2002; Dale and Batal, 2003 and Shurson *et al.*, 2004).

Distiller's grain contained 0.2 to 0.5 per cent of sulphur (Spiehs *et al.*, 2002 and Shurson *et al.*, 2004), 120 to 150 ppm of iron (Shelford and Tait, 1986 and Spiehs *et al.*, 2002), 60 to 100 ppm of zinc (Shelford and Tait, 1986 and Spiehs *et al.*, 2002), 15 to 25 ppm of copper (Shelford and Tait, 1986) and 15 ppm of manganese (Spiehs *et al.*, 2002). Dale and Batal (2003) reported higher sulphur content (0.84 per cent), Spiehs *et al.* (2002) reported a lower copper content (5.9 ppm) and Shelford and Tait (1986) reported a higher manganese content (66 ppm) in distiller's grains.

2.2.3 Nutritive Value of Brewer's Grain and Distiller's Grain

The TDN content of brewer's grain and distiller's grain was 70 to 80 per cent as reported by Crickenberger and Johnson (1982) and Depeters *et al.* (1997). Lower TDN value of 48.2 per cent was reported by Hayashi *et al.* (2005).

Edionwe and Owen (1989) observed that the TDN content of corn distiller's grain was 66.6 per cent, whereas Schroeder (2003) reported 86 and 88 per cent of TDN in distiller's grain and distiller's grain plus solubles, respectively. Garcia and Kalscheur (2004) observed that the TDN content of distiller's grain was 79.5 per cent. Russ *et al.* (2005) reported that net and gross calorific values of brewer's spent grain were 18.64 MJ/kg and 20.14 MJ/kg of DM, respectively.

The rumen undegradable protein (RUP) content of distiller's grain and brewer's grain was 45 to 55 per cent of CP as reported by Shaver (1989) and Belibasakis and Tsirgogianni (1996), respectively. Higher RUP value of 71 per cent of CP was reported by Dhiman *et al.* (2003).

2.3 PRESERVATION OF BREWER'S GRAIN AND DISTILLER'S GRAIN

Shelf life of wet brewer's grain and distiller's grain was only 4 to 5 days (Rendell, 2004). Russ *et al.* (2005) reported that due to its fermentable sugars and high moisture contents, brewer's spent grain is a very unstable material and get deteriorated rapidly due to microbial activity.

2.3.1 Physical Method

2.3.1.1 Drying

Sun drying of grain is the most common preservation method in tropical developing countries. Chancellor (1965) reported that drying on flat exposed surfaces is the most common way of drying grain. Santos *et al.* (2003) reported that drying of brewer's spent grain reduce the volume and decrease the transport and storage costs.

Oven drying of brewer's spent grain must be conducted at temperatures below 60^oC, because at higher temperatures unpleasant flavours are generated (Prentice and Appolonia, 1977 and Hernandez *et al.*, 1999) and create odour pollution problems (Huige, 1994). Santos *et al.* (2003) reported that oven drying at 60^oC for 18 hours could preserve brewer's spent grains effectively.

Bartolome *et al.* (2002) evaluated brewer's spent grain by various preservation methods such as freeze-drying, oven drying and freezing and found that freezing was inappropriate due to its bulkiness. Preservation by oven drying or freeze drying reduces the volume of the product without altering its composition. However, freeze drying was economically unacceptable. Tang *et al.* (2005) reported that steam drying (superheated steam) of brewer's spent grain could save energy which claimed additional advantages including improved drying efficiency, elimination of fire or explosion risk and enhanced recovery of valuable organic compounds.

2.3.1.2 Addition of Adsorbents

Sansoucy *et al.* (1988) used rice bran as an adsorbent in making nutrient feed blocks. Wet distiller's grain was preserved by ensiling with soya hulls, by combining wet distiller's grain with soya hulls in the ratio of 30:70 (Garcia and Kalscheur, 2004).

2.3.2 Chemical Method

2.3.2.1 Organic Acids

Muller and Thaler (1981) reported that addition of propionic acid at levels of 0.3, 0.5, 0.7 and 1.0 per cent to corn having moisture content of 19, 25, 32 and 40 per cent, respectively inhibited the growth of inoculated fungi during storage for at least 6 months at 20^{o}C. Propionic acid is an organic acid that acts as fungicide inhibiting growth of aerobic microorganisms that can cause heating and moulding. Al-Hadithi *et al.* (1985) reported that combination of formic or benzoic acid–water– brewer's spent grain mixture packed in plastic containers, held for three summer months, preserved the quality and nutritional value of brewer's spent grain. Rahnema and Neal (1994) reported that propionic acid treatment effectively inhibited carbon dioxide production for 13 and 9 days with corn containing 15 and 18 per cent moisture, respectively. Bagg (2004) reported that propionic acid is the most effective acidic mould inhibitor. Kung *et al.* (2004) reported that buffered propionic acid based additives improved the fermentation and aerobic stability of high moisture corn silages. On the contrary, Kleinschmit *et al.* (2005) reported that buffered propionic acid additive did not improve the aerobic stability and DM recovery of silage.

2.4 *IN VITRO* STUDIES USING RUSITEC

RUSITEC is an instrument first developed and described by Czerkawski and Breckenridge (1977) for simulating rumen fermentation under strictly controlled conditions. RUSITEC is a rumen without rumen wall and it is an *in vitro* semi continuous culture system. The technique was further developed to overcome the existing problems in other *in vitro* techniques of Carro and Miller (1998) and Covaldes *et al.* (1998).

2.4.1 Sample Size to Bag Surface Ratio

The ideal sample size to bag surface ratio had been quoted as about 15 mg dry matter/cm^2. The incubated sample should be able to move freely within the bags to avoid formation of microenvironments that affect replication of the analysis (Orskov, 1992 and Michalet and Oulhbah, 1992).

2.4.2 Bag Pore Size

Carro *et al.* (1995) reported that pore size of the nylon bags in RUSITEC affects not only the disappearance of the diet, but also the microbial population in the system and fermentation. They reported that the disappearance of the diet from the nylon bags, methane and acetate production in RUSITEC were higher with 100 μm bag pore size than with those of 40 μm and 200 μm pore size.

2.4.3 Sample Preparation

Michalet and Oulhbah (1992) reported that the dry sample should be milled and sieved through a 2.5 to 3.0 mm diameter sieve, while the green forages and silage should be processed using a mincer with 3 mm screen to reproduce the effect of chewing.

2.4.4 Stoichiometry for Rumen Fermentation

2.4.4.1 Acetate to Propionate Ratio

Czerkawski (1986) reported that high forage diet produced acetate, propionate and butyrate in the ratio of 60:30:10 respectively indicating higher acetate: propionate (A:P) ratio. He further reported that with high grain ration, ratio of acetate, propionate and butyrate was 45:40:10 indicating lower acetate to propionate ratio.

2.4.4.2 Non Glucogenic Ratio (NGR)

Orskov (1975) proposed the concept of NGR of metabolites entering intermediary metabolism. He defined it as the total amount of substrate absorbed which cannot yield glucose (or) glucose precursors relative to the total amount of substance, which is observed as glucose and can give rise to a net synthesis of glucose.

$$\text{NGR} = \frac{\text{Acetate} + (2 \text{ x butyrate})}{\text{Propionate}}$$

He also suggested that an NGR value of 2.25 to 3.00 would result in the highest efficiency for growth and fattening. Orskov (1977) concluded that higher the NGR, the higher would be the production of methane. The formula for NGR was modified (Mc Donald *et al.*, 2002) as

$$= \frac{\text{Acetate} + (2 \text{ x Butyrate}) + \text{Valerate}}{\text{Propionate} + \text{Valerate}}$$

2.5 RUMEN DEGRADABILITY

2.5.1 *In Situ* Degradability

2.5.1.1 Dry Matter

Davis *et al.* (1983) reported that the *in situ* DM disappearance rate was higher for wet brewer's grain than the dried brewer's grains. Firkins *et al.* (1985) studied the *in situ* DM disappearance of wet and dry corn distiller's grain using four rumen cannulated steers and reported values of 3.4 and 2.8 per cent per hour respectively. Orskov *et al.* (1992) reported ruminal *in situ* disappearance of DM of brewer's grain as 53.8 per cent. The *in sacco* DM degradability of different varieties of paddy straw at 72 hours of incubation ranged from 42.76 to 59.67 per cent (Sohane and Singh, 2000). Reddy and Sivaiah (2001) also reported *in sacco* DM degradability for semi dwarf and medium varieties of paddy straw as 60.11 and 60.27 per cent, after 72 hours of incubation, respectively.

Mustafa *et al.* (2000a) reported that the *in situ* effective ruminal degradability of DM in cows was higher for wheat distiller's grain (521.8 g/kg) than for barley distiller's grain (439.2 g/kg). Mustafa *et al.* (2000b) reported that *in situ* effective ruminal degradability of DM in non-lactating Holstein cows was highest for rye distiller's grain (528 g/kg) and lowest for barley distiller's grain (381 g/kg). Ruminal degradability of DM was higher for triticale (474 g/kg) than for wheat distiller's grain (431 g/kg).

Trach (2003b) studied the 48 hour *in situ* DM degradability of rice straw based diet supplemented with 10 per cent wet brewer's grains in fistulated animals and found significant improvement due to the addition of wet brewer's grain from 48.3 to 55.4 per cent. He further reported that supplementation of 10 per cent wet brewer's grains to treated (3 per cent lime plus 2 per cent urea) rice straw significantly increased the 48 hour DM degradability from 50.9 to 56.4 per cent in fistulated animals.

The *in situ* DM disappearance at 24 hours of incubation from gingelly oil cake, groundnut cake, cotton seed cake and coconut cake were 90.83, 79.20, 61.95 and 60.52 per cent, respectively in fistulated Jersey crossbred cows (Kalbande, 1995). He further reported that *in situ* DM disappearance of green fodder such as maize, jowar, paragrass, napier grass and guinea grass at 48 hours of incubation were 76.21, 77.43, 57.05, 64.41 per cent, respectively and the *in situ* DM disappearance of energy feeds such as yellow maize, jowar, wheat bran, rice bran and tapioca flour at 24 hours of incubation were 63.43, 62.50, 64.90, 56.13 and 91.32 per cent, respectively.

2.5.1.2 Protein

Davis *et al.* (1983) reported that, wet brewer's grain, in contrast to the dried grains showed a higher disappearance rate of CP. Carroll *et al.* (1997) reported that *in situ* undegraded intake protein value of barley distiller's grain and corn distiller's grain in lactating Holstein cows ranged from 37 to 44 and 42 to 44 per cent of CP, respectively. Mustafa *et al.* (2000b) reported that *in situ* effective ruminal protein degradability in non-lactating Holstein cows was higher for wheat (539 g/kg) and rye (541 g/kg) distiller's grain than for barley distiller's grain (492 g/kg). The ruminal escape protein value was higher for barley distiller's grain (490 g/kg of CP) than for wheat distiller's grain (414.5 g/kg) (Mustafa *et al.*, 2000a). They reported that *in situ* effective ruminal protein degradability in cows was lower for barley distiller's grain (616 g/kg) than for wheat distiller's grain (651.1 g/kg). Peter *et al.* (2000) reported that CP degradation of corn distiller's grain in ruminally cannulated beef steers was 39.2 per cent at 0 hour and 57.6 per cent at 48 hours.

The *in situ* nitrogen disappearance at 24 hours of incubation from gingelly oil cake, groundnut cake, cotton seed cake and coconut cake were 92.06, 89.82, 38.30 and 29.14 per cent, respectively in fistulated Jersey crossbred cows (Kalbande, 1995). He further reported *in situ* nitrogen disappearance of green fodder such as maize, jowar, paragrass, napier grass and guinea grass at 48 hours of incubation as 77.75, 78.26, 64.33, 75.24 and 67.66 per cent, respectively and of energy feeds such as yellow maize, jowar, wheat bran, rice bran and tapioca flour at 24 hours of incubation as 35.79, 61.08, 89.45, 72.66 and 87.16 per cent, respectively.

2.5.1.3 Fibre

The *in situ* NDF disappearance rates for wet and dry corn distiller's grain in rumen cannulated steers were reported to be 4.4 and 3.7 per cent per hour respectively (Firkins *et al.*, 1985). Batajoo and Shaver (1998) reported that 24 hour *in situ* NDF disappearance was 33.3 per cent for brewer's dried grain and 43.6 per cent for distiller's dried grain. Mustafa *et al.* (2000b) reported that *in situ* effective degradability of NDF in non-lactating Holstein cows was highest for rye distiller's grain (470 g/kg) followed by wheat (450 g/kg) and triticale distiller's grain (439.00 g/kg) and lowest for barley distiller's grain (342 g/kg). Mustafa *et al.* (2000a) reported that *in situ* effective degradability of NDF in cows was lower for barley distiller's grain (359.8 g/kg) relative to wheat distiller's grain (454 g/kg).

2.5.2 *In Vitro* Degradability

2.5.2.1 Dry Matter

Carro *et al.* (1992) reported that high concentrate diet having a forage to concentrate ratio of 30:70 with yeast culture significantly increased the *in vitro* DM degradability compared to other two diets having a forage to concentrate ratio of 70:30 and 50:50. Treatment of coastal bermuda grass with fibrolytic enzymes had no effect on *in vitro* DM degradability (IVDMD) and *in vitro* NDF disappearance after 48 hours of incubation (Mandebvu *et al.*, 1999). However, Liu and Orskov (2000) observed that cellulase (16 units per gram DM for 3 weeks) treatment of steam pretreated rice straw significantly increased the 24 hour *in vitro* organic matter degradability than that of untreated rice straw.

Reddy and Sivaiah (2001) reported that the IVDMD of semi dwarf and medium varieties of paddy straw was 28.03 and 29.26 per cent, respectively after 48 hours of incubation done as per Tilley and Terry (1963) method.

Reed *et al.* (2006) reported that the *in vitro* organic matter degradability (IVOMD) of corn distiller's dried grains was significantly reduced (79.33 per cent) compared to control diet containing soyabean meal and wheat middlings (85.67 per cent) done as per Tilley and Terry (1963) method.

2.5.2.2 Protein

Sehgal and Makkar (1994) reported that the rumen *in sacco* protein degradability of groundnut cake in growing buffalo calves was 96.3 per cent, whereas *in vitro* protein degradability was 77.9 per cent as per Tilley and Terry (1963) method. They further reported that the effective CP degradability of groundnut cake was 81.9 per cent at an outflow rate of 0.05 per hour.

2.5.2.3 Fibre

Carro *et al.* (1992) reported that high concentrate diet (forage to concentrate ratio of 30:70) with yeast culture significantly increased the *in vitro* NDF degradability than those fed without yeast culture.

Nsereko *et al.* (2000) reported that pretreatment of alfalfa hay with fibrolytic enzymes (polysaccharidase and glycosidase) for 2 hours significantly increased the *in vitro* NDF degradability by 24 per cent at 12 hours of incubation. Similarly, hemicellulose degradation was also increased but the degradation was highest only at 48 hours of incubation *in vitro*.

2.5.3 RUSITEC Degradability

2.5.3.1 Dry Matter

Durand *et al.* (1988) observed a significant improvement in the *in vitro* (RUSITEC) organic matter degradability when wheat straw was treated with sodium hydroxide. Similarly, addition of cationomycin (Ionophore) to fescue hay based pelleted diet showed increased *in vitro* (RUSITEC) DM degradability (Bogaert *et al.*, 1989). However, no significant difference was observed in the 48 hour *in vitro* (RUSITEC) DM degradability of alfalfa or wheat straw as a result of monensin addition (Bogaert *et al.*, 1990). Carro *et al.* (1995) found significant improvement in the *in vitro* (RUSITEC) DM disappearance of grass and corn silage at 48 hours of incubation when nylon bags with pore size of 100 μm was used, compared to those with 40 μm pore size. Addition of fibrolytic enzymes (183048 IU of cellulase and 46288 IU of xylanase per kg DM for dry grass and 30000 nova cellulase and 10000 nova xylanase units per kg DM for orchard grass hay) improved the *in vitro* (RUSITEC) DM disappearance at 48 hours incubation of dry grass (Feng *et al.*, 1996) and orchard grass hay (Dong *et al.*, 1999).

Wang *et al.* (1998) studied the *in vitro* DM disappearance of alfalafa hay and concentrate after adding *Yucca schidigera* extract using RUSITEC and found no significant difference due to addition of *Yucca schidigera* extract. Wang *et al.* (2001) could not observe any significant improvement in the *in vitro* (RUSITEC) DM and OM degradability when barley straw was mixed with four nitrogen supplements such as fish meal, casein, soyabean meal and urea. Similarly, Sliwinski *et al.* (2002) also could not observe any significant difference in the 48 hour *in vitro* (RUSITEC) OM degradability of basal diet (grass silage, barley grain and grass hay) compared with soyabean meal based diet. Hess *et al.* (2003) studied the *in vitro* OM degradability of hay and barley straw based diet with faunated and defaunated group using RUSITEC at 48 hours incubation and found no significant difference between them.

2.5.3.2 Protein

Wang *et al.* (2001) found significant improvement in the *in vitro* (RUSITEC) nitrogen degradability when barley straw was mixed with four nitrogen supplements such as fish meal, casein, soyabean meal and urea. Hess *et al.* (2003) observed a significant improvement in the *in vitro* (RUSITEC) CP degradability of hay and barley straw based diet in the defaunated group (54 per cent) compared to faunated group (49 per cent).

Bogaert *et al.* (1989) found addition of cationomycin (Ionophore) to fescue hay based pelleted diet did not improve the *in vitro* (RUSITEC) nitrogen degradability. Similarly, Sliwinski *et al.* (2002) found no significant difference in the *in vitro* (RUSITEC) CP degradability of barley grain and soyabean meal based diet.

2.5.3.3 Fibre

Durand *et al.* (1988) observed a significant improvement in the *in vitro* (RUSITEC) hemicellulose and cellulose degradability when wheat straw was treated with sodium hydroxide. Similarly, addition of cationomycin (Ionophore) to fescue hay based pelleted diet showed increased *in vitro* (RUSITEC) NDF, ADF, hemicellulose and cellulose degradability (Bogaert *et al.*, 1989). Bogaert *et al.* (1990) found significant improvement in the *in vitro* (RUSITEC) hemicellulose degradability of alfalfa or wheat straw as a result of monensin addition. Carro *et al.* (1995) found significant improvement in the *in vitro* (RUSITEC) NDF disappearance of grass and corn silage when nylon bags with pore size of 100 μm was used, compared to those with 40 μm pore size. Addition of fibrolytic enzymes (30000 nova cellulase and 10000 nova xylanase units per kg DM) significantly improved the *in vitro* (RUSITEC) cellulose and hemicellulose disappearance of orchard grass hay (Dong *et al.*, 1999). Wang *et al.* (2001) also found significant improvement in the *in vitro* (RUSITEC) NDF degradability of barley straw when mixed with nitrogen supplements such as fish meal and soyabean meal compared to the urea supplementation. Significant improvement was observed in the *in vitro* (RUSITEC) NDF and ADF degradability of *Vigna unguiculata* when mixed with a tropical grass *Brachiaria humidicola*, compared to those with *Leucaena leucocephala* (Hess *et al.*, 2007).

However, significant reduction was observed in the *in vitro* (RUSITEC) NDF, ADF and cellulose degradability of alfalfa as a result of monensin addition (Bogaert *et al.*, 1990).

Sliwinski *et al.* (2002) found no significant difference in the 48 hour *in vitro* (RUSITEC) NDF, ADF and hemicellulose degradability of basal diet (containing grass silage, barley grain and grass hay) compared to those with soyabean meal based diet. No significant difference was observed in the 48 hour *in vitro* (RUSITEC) NDF degradability of hay and barley straw based diet as a result of defaunation (Hess *et al.*, 2003).

2.5.4 Potential / Effective Degradability

Orskov and McDonald (1979) reported that potential degradability of nutrients were given by the formula, P (rumen degradability at time t) $= a + b\,(1–e^{-ct})$. McDonald *et al*. (2002) modified this formula to calculate effective degradability $P = a + \{bc/(c+r)\}$.

Kalbande (1995) found *in situ* effective protein degradability of gingelly oil cake, groundnut cake, cotton seed cake and coconut cake as 78.99, 66.17, 27.72 and 19.93 per cent, respectively. He further reported that the *in situ* effective protein degradabilities of green fodder such as maize, jowar, paragrass, napier grass and guinea grass were 56.93, 58.80, 45.39, 36.68 and 35.74 per cent, respectively and that of energy feeds such as yellow maize, jowar, wheat bran, rice bran and tapioca flour were 22.64, 47.96, 74.16, 60.59 and 68.83 per cent, respectively.

2.5.4.1 Soluble Fraction ‘a’

Aman and Hesselman (1984) reported that the ‘*a*’ fraction contains soluble sugars such as glucose, fructose, sucrose and fructans, as well as soluble non starch polysaccharides arabinose, xylose, mannose, galactose and uronic acids. Differences observed in this fraction among feeds could also be due to the variation in feed particle size. Small feed particles could be physically expelled from the bag during soaking prior to incubation and washing which would over estimate the amount leaving the bag due to solubilization. Also, soluble protein or particles exiting the bag do not necessarily reflect degradation. For example, bovine serum albumin, which is soluble in water, is not highly degradable when incubated in rumen liquor (Broderick, 1987 and Broderick and Craig, 1989).

Orskov *et al.* (1992) reported the '*a*' fraction for ruminal *in situ* effective degradability of DM of brewer's grain as 15.2 per cent. Batajoo and Shaver (1998) reported that DM '*a*' fraction was highest for distiller's dried grain (40.2 per cent) and lowest for brewer's dried grain (14.5 per cent). They further reported that CP '*a*' fractions were similar for brewer's dried grain and distiller's dried grain (17 per cent) and starch '*a*' fraction was highest for distiller's dried grain (77.7 per cent) and intermediate for brewer's dried grain (59 to 68 per cent). Reddy and Sivaiah (2001) reported that there was a significant difference in '*a*' fraction between fifteen varieties of paddy straw (semi dwarf and medium) and it ranged from 8.33 to 14.06 per cent. Sohane and Singh (2001) observed the rapidly soluble '*a*' fraction of paddy straw as 14.1 per cent.

2.5.4.2 Insoluble but Potentially Degradable Fraction 'b'

Orskov *et al.* (1992) reported that ruminal *in situ* effective degradability of DM '*b*' fraction of brewer's grain was 50.7 per cent. Batajoo and Shaver (1998) reported that DM '*b*' fraction was 57.2 per cent for brewer's dried grain and 44 per cent for distiller's dried grain. They further reported that CP '*b*' fraction for brewer's dried grain was 64.3 per cent and that for distiller's dried grain was 56.1 per cent and starch '*b*' fraction was 23.4 per cent for brewer's dried grain and 16.7 per cent for distiller's dried grain. Reddy and Sivaiah (2001) reported that there was a significant difference in '*b*' fraction between fifteen varieties of paddy straw (semi dwarf and medium) which ranged from 51.75 to 56.97 per cent, while Sohane and Singh (2001) reported the degradable fraction of paddy straw as 35.05 per cent. Trach (2003b) reported similar dry matter '*b*' fraction of 47.1 and 47 per cent in untreated and treated rice straw (3 per cent lime + 2 per cent urea) with 10 per cent wet brewer's grain using rumen fistulated animals.

2.5.4.3 Rate of Degradation (c)

Orskov *et al.* (1992) reported that ruminal *in situ* effective degradability of DM '*c*' fraction of brewer's grain was 0.0282/hour. Batajoo and Shaver (1998) reported similar DM degradation rates (c) of 0.049/hour for brewer's dried grain and distiller's dried grain.

Trach (2003b) reported DM '*c*' fraction of untreated and treated rice straw (3 per cent lime + 2 per cent urea) when supplemented with 10 per cent wet brewer's grain were significantly higher (0.040 and 0.042/hour respectively) compared to those with non-supplemented (0.035 and 0.030/hour respectively) rumen fistulated animals.

Batajoo and Shaver (1998) reported that rate of CP degradation was 0.06 to 0.07/hour for brewer's dried grain and 0.04 to 0.05/hour for distiller's dried grain. Grings *et al.* (1992) reported a similar CP degradation rate of 0.049/hour for distiller's dried grain, but Nocek (1987) reported a slightly faster CP degradation rate of 0.068/h.

Batajoo and Shaver (1998) reported the rate of starch degradation in brewer's dried grain as 0.21 to 0.27/hour and that of starch degradation of distiller's dried grain as 0.10 to 0.14/hour.

Colombatto (2000) stated that treatment of forage with fibrolytic enzyme mixtures increased the rate of degradation *in vitro*. Reddy and Sivaiah (2001) reported that there was a significant difference of '*c*' fraction between fifteen varieties of paddy straw (semi dwarf and medium) which ranged from 0.0352 to 0.0441 per hour. Sohane and Singh (2001) also reported similar degradation rate (c) of 0.03 per hour for paddy straw.

2.5.4.4 Rate of Passage (r)

Batajoo and Shaver (1994) reported that rate of passage was assumed at 0.07/hour for calculation of ruminal availabilities.

2.5.4.5 Lag Time (L)

Trach (2003b) reported lag time (L) of untreated and treated rice straw (3 per cent lime + 2 per cent urea) when supplemented with 10 per cent wet brewer's grain was (3 and 3.6 hour respectively) significantly lower compared to those with non-supplemented (4.3 and 4.6 hour respectively) rumen fistulated animals.

2.6 RUMEN FERMENTATION PARAMETERS

2.6.1 pH

2.6.1.1 Rusitec

Wang *et al.* (2001) found significant difference in pH when barley straw was mixed with four nitrogen supplements such as fish meal, casein, soyabean meal and urea, values being 6.58, 6.56, 6.56 and 6.83, respectively using RUSITEC. Tejido *et al.* (2002) found significant difference in pH when barley straw was fed to sheep (6.20 and 5.79) vs RUSITEC (6.73 and 6.04) in the diets containing forage to concentrate ratio of 80:20 and 20:80 based diets respectively.

However, no significant difference was observed in the RUSITEC fluid pH of fescue hay based pelleted diet as a result of cationomycin (Ionophore) addition (Bogaert *et al.*, 1989). Dong *et al.* (1999) found pretreatment of orchard grass hay with fibrolytic enzymes (30000 nova cellulase and 10000 nova xylanase units per kg DM) did not influence pH of RUSITEC liquor. Hess *et al.* (2003) found no significant difference in the RUSITEC fluid pH of hay and barley straw based diet as a result of defaunation. Hess *et al.* (2007) found no significant difference in RUSITEC fluid pH when *Vigna unguiculata* mixed with a tropical grass *Brachiaria humidicola* or *Leucaena leucocephala.*

Carro *et al.* (1995) found significant reduction in the RUSITEC fluid pH of grass and corn silage based diet when nylon bags with pore size of 100 μm was used, compared to those with 40 μm pore size.

2.6.1.2 In Situ

Kalbande (1995) observed significant difference in pH of rumen liquor at 3, 6, 9 and 12 hours of incubation with concentrate mixtures containing RDP to UDP ratio of 40:60, 55:45 and 65:35 in fistulated animals.

Trach (2003b) studied the pH of rumen liquor in fistulated animals fed rice straw based diet supplemented with 10 per cent wet brewer's grains and found significant reduction due to the addition of wet brewer's grain from 7.09 to 6.53. He further reported that supplementation of 10 per cent wet brewer's grains to treated rice straw (3 per cent lime + 2 per cent urea) significantly reduced the pH from 6.71 to 6.34 in fistulated animals.

2.6.1.3 In Vivo

Murdock *et al.* (1981) found rations containing wet brewer's grain had no effect on rumen pH. Davis *et al.* (1983) found significant difference in the pH of rumen liquor when fed 20, 30 and 40 per cent of pressed brewer's grain supplemented diets (6.2, 6.2 and 6.5, respectively) compared to those with non supplemented diet (6.0). Ruminally cannulated crossbred beef steers fed corn distiller's dried grains showed a ruminal pH of 6.24 (Peter *et al.,* 2000), whereas Al-Suwaiegh *et al.* (2002) reported a value of 6.3. Ally (2003) reported that the pH of rumen liquor as 6.42 and 6.57 in cows fed with rations containing undegradable protein levels of 26.8 and 42.9 per cent, respectively.

2.6.2 Ammonia Nitrogen (NH_3-N)

2.6.2.1 Rusitec

Wang *et al.* (2001) found significant difference in the *in vitro* (RUSITEC) NH_3-N concentration when barley straw was mixed with four nitrogen supplements such as fish meal, casein, soyabean meal and urea (9.1, 17.9, 14.5 and 22.8 mg/100 ml, respectively). Tejido *et al.* (2002) also found significant difference in the NH_3-N concentration of RUSITEC fluid for barley straw (289 and 250 mg/l) in the forage to concentrate ratio of 80:20 and 20:80, respectively. Hess *et al.* (2003) found significant reduction in the RUSITEC fluid NH_3-N concentration of hay and barley straw based diet (from 8.5 to 5.9 mmol/l) as a result of defaunation.

Carro *et al.* (1995) found no significant difference in the NH_3-N concentration of RUSITEC fluid for grass and corn silage based diet, when nylon bags with pore size of 100 and 40 μm were used. Wang *et al.* (1998) also did not observe any significant difference in the 72 hour NH_3-N concentration for alfalafa hay based diet after adding *Yucca schidigera* extract using RUSITEC.

2.6.2.2 In Situ

Kalbande (1995) observed significant difference in NH_3-N concentration of rumen liquor at 3, 6, 9 and 12 hours incubation with concentrate mixtures containing RDP to UDP ratio of 40:60, 55:45 and 65:35 in fistulated animals.

Trach (2003b) found no significant difference in rumen liquor NH_3-N concentration of untreated and treated rice straw (3 per cent lime + 2 per cent urea) based diet supplemented with 10 per cent wet brewer's grains were 266 and 244 mg/l respectively in fistulated animals.

2.6.2.3 In Vivo

Ham *et al.* (1994) found NH_3-N concentration of 9.51 mg/100 ml of rumen liquor of beef cattle heifers, whereas, Reed *et al.* (2006) could observe lower NH_3-N concentration of 5.67 mg/100ml of rumen liquor of steer calves fed with wet corn distiller's grain.

Anderson *et al.* (2006) found significant reduction in NH_3-N concentration of cows when fed with 20 per cent dried distiller's grain solubles based diet compared to those with 10 per cent dried distiller's grain solubles based diet. They further observed that the cows fed with 10 per cent wet distiller's grain solubles based diet had lower NH_3-N concentration than the cows fed with 20 per cent wet distiller's grain solubles based diet. Anderson *et al.* (2006) found that concentration of NH_3-N was less than observed in other research with distiller's grain (Nichols *et al.*, 1998; Schingoethe *et al.*, 1999 and Liu *et al.*, 2000).

Ally (2003) reported that NH_3-N concentration of rumen liquor was 36.42 and 27.27 mg/100 ml in cows fed with rations containing undegradable protein levels of 26.8 and 42.9 per cent, respectively.

2.6.3 Total Volatile Fatty Acids (TVFA)

2.6.3.1 Rusitec

Durand *et al.* (1988) observed a significant improvement in the *in vitro* (RUSITEC) TVFA concentration of 40.5 to 61.1 mmol/day, when wheat straw was treated with sodium hydroxide. Wang *et al.* (2001) found significant difference in TVFA concentration using RUSITEC, when barley straw was mixed with four nitrogen supplements such as fish meal, casein, soyabean meal and urea (113.5, 121.2, 131.5 and 100.3 mmol/l respectively). Hess *et al.* (2003) found significant reduction in the TVFA concentration (from 85.4 to 78.5 mmol/l) of barley straw based diet using RUSITEC as a result of defaunation.

Bogaert *et al.* (1989) found no significant difference in the TVFA concentrations of fescue hay based pelleted diet using RUSITEC as a result of cationomycin (Ionophore) addition. Carro *et al.* (1995) observed no significant difference in the TVFA concentrations of grass and corn silage based diet when nylon bags with pore size of 100 and 40 μm was used in RUSITEC. Wang *et al.* (1998) did not find any significant difference in the 72 hour TVFA concentration of alfalafa hay based diet after adding *Yucca schidigera* extract in RUSITEC fluid. Dong *et al.* (1999) studied the TVFA concentration of orchard grass hay pre treated with fibrolytic enzymes (30000 nova cellulase and 10000 nova xylanase units per kg DM) using RUSITEC and found no significant difference due to the addition of fibrolytic enzymes. No significant difference in the TVFA concentrations of barley straw fed to RUSITEC system (Tejido *et al.*, 2002) and the values reported were 93.8 and 95.0 mmol/l in the forage to concentrate ratio of 80:20 and 20:80 based diet respectively. Hess *et al.* (2007) found no significant difference in the RUSITEC liquor TVFA concentration of *Vigna unguiculata* mixed with a tropical grass *Brachiaria humidicola* and *Leucaena leucocephala*.

2.6.3.2 In Situ

Kalbande (1995) observed significant difference in TVFA concentration of rumen liquor at 3, 6 and 12 hours of incubation with concentrate mixtures (RDP to UDP ratio of 40:60, 55:45 and 65:35) in fistulated animals.

Trach (2003b) found significant improvement in TVFA concentration from 68 to 94 mmol/l due to addition of 10 per cent wet brewer's grains to rice straw based diet in fistulated animals. He further reported that supplementation of 10 per cent wet brewer's grains to treated (3 per cent lime plus 2 per cent urea) rice straw significantly increased the TVFA concentration from 93 to 114 mmol/l in fistulated animals.

2.6.3.3 In Vivo

Varel and Kreikemeier (1994) found significant improvement in TVFA concentration in the rumen of cows when fed with brome grass as a result of Amafern (*Aspergillus oryzae*) addition. Hristov *et al.* (1998) found fibrolytic enzyme treatment significantly improved the rumen TVFA concentration of total mixed ration in heifers. Huang *et al.* (1999) found significant improvement in TVFA concentration in the rumen of cows when fed with 10 per cent rice distiller's grain (88.16 mmol/l) compared to those with 20 per cent inclusion (78.17 mmol/l). Similarly, Beauchemin *et al.* (2000) observed significant improvement in

TVFA concentration in rumen of heifers when total mixed ration was treated with fibrolytic enzymes.

Kamra *et al.* (2002) found no significant difference in TVFA concentration of rumen liquor as a result of yeast culture supplementation to the cows. No significant difference in rumen TVFA concentration of cows when fed with diets containing 10 and 20 per cent of dried distiller's grains and wet distiller's grains (Al-Suwaiegh *et al.*, 2002 and Anderson *et al.*, 2006).

Davis *et al.* (1983) found significant reduction in TVFA concentration of rumen liquor of cows when fed with diets containing 20, 30, 40 per cent pressed brewer's grain based diets (99, 98 and 85 mmol/l, respectively) compared to those with control diet (113mmol/l). Similarly, significant reduction in rumen TVFA concentration was observed when the cows were fed with diets containing distiller's grains (Nichols *et al*., 1998 and Schingoethe *et al*., 1999).

Ally (2003) reported that cows fed with rations containing undegradable protein levels of 26.8 and 42.9 per cent had rumen TVFA concentration of 76.10 and 77.60 meq/l, respectively.

2.6.4 Volatile Fatty Acid (VFA) Fractions

2.6.4.1 Rusitec

Carro and Miller (1999) studied the effect of different nitrogen forms on a fibre based diet using RUSITEC and the production of acetate, propionate and butyrate were 26.14, 26.81 and 2.45 mmol/l for soyabean meal. Wang *et al.* (2001) observed significant difference in acetate, propionate, isobutyrate, butyrate, isovalerate and valerate concentration using RUSITEC, when barley straw was mixed with four nitrogen supplements such as fish meal (68.5, 20.5, 0.9, 7.1, 0.57 and 1.4 molar per cent), casein (65.7, 21.4, 1.4, 7.3, 1.34 and 1.6

molar per cent), soyabean meal (67.9, 19.5, 1.0, 8.3, 0.87 and 1.3 molar per cent) and urea (69.9, 20.8, 0.5, 7.4, 0.20 and 0.8 molar per cent), respectively**.** Hess *et al.* (2003) found significant difference in the molar proportion of acetate, propionate, butyrate and valerate concentration of hay and barley straw based diet using RUSITEC as a result of defaunation (0.531, 0.313, 0.099 and 0.0435 mmol/l, respectively) when compared with faunated group (0.597, 0.248, 0.114 and 0.0276 mmol/l, respectively).

No significant difference was observed in molar proportion of acetate, propionate, butyrate and valerate concentration using RUSITEC, when wheat straw was treated with ammonia and alkali (Durand *et al*., 1988). Similarly, addition of cationomycin (Ionophore) to fescue hay based pelleted diet showed no significant difference in the acetate and isobutyrate concentration, but significant improvement was observed in the propionate, butyrate and valerate concentration as a result of cationomycin supplementation (26.0, 5.81 and 2.50 mmol per day, respectively) compared to those with non supplemented group (16.71, 8.65 and 1.46 mmol per day, respectively) using RUSITEC (Bogaert *et al.*, 1989). Carro *et al.* (1995) found no significant difference in acetate, butyrate and valerate concentration of grass and corn silage based diet using RUSITEC, when nylon bags with pore size of 100 and 40 μm was used. However, significant improvement in propionate concentration was observed when nylon bags with pore size of 40 μm were used (19.7 molar per cent), compared to those with 100 μm pore size (18.2 molar per cent).

Wang *et al.* (1998) studied the molar proportion of acetate, propionate and butyrate of alfalafa hay based diet after adding *Yucca schidigera* extract using RUSITEC and found no significant difference due to the addition of *Yucca schidigera* extract. Similarly, no significant difference was observed in the acetate and propionate concentration of orchard grass hay pretreated with fibrolytic enzymes (30000 nova cellulase and 10000 nova xylanase units per kg DM) using RUSITEC, but there was significant improvement in the butyrate concentration as a result of pretreatment of orchard grass hay with fibrolytic enzymes (21.4 molar per cent) compared to those with non treated orchard grass hay (18.2 molar per cent) (Dong *et al.*, 1999). Hess *et al.* (2007) found no significant difference in the molar proportion of acetate, propionate, butyrate concentaration and A:P ratio of *Vigna unguiculata* mixed with a tropical grass *Brachiaria humidicola* and *Leucaena leucocephala* using RUSITEC.

2.6.4.2 In Situ

Yoon and Stern (1996) found supplementation of mixture of yeast (*Saccharomyces cerevisiae*) and fungal (*Aspergillus oryzae*) cultures significantly reduced the molar per cent of ruminal volatile fatty acids in fistulated animals.

Flachowsky *et al.* (1990) found significant increase in TVFA concentration of rumen liquor and molar concentration of propionate and butyrate, but decreased acetate with high concentrate feeding (such as roughage concentrate ratio of 50: 50 and 25: 50) in fistulated sheep.

Kalbande (1995) observed significant difference in acetate, propionate and butyrate concentration of rumen liquor at 3, 6 and 12 hours incubation with concentrate mixtures (containing RDP to UDP ratio of 40:60, 55:45 and 65:35) in fistulated animals.

2.6.4.3 In Vivo

Murdock *et al.* (1981) studied the volatile fatty acid fractions of diets containing wet brewer's grains as a concentrate replacement at 15 and 30 per cent of the total ration DM in lactating dairy cows and found that molar per cent of acetate, propionate and butyrate concentration was 60.9 vs 62.2, 23.6 vs 22.0 and 15.5 vs 15.8 respectively and A:P ratio was 2.7 and 2.9, respectively for diets containing wet brewer's grain as a concentrate replacement at 15 and 30 per cent of the total ration DM. They found no effect on molar ratio of volatile fatty acids when cows were fed with rations containing wet brewer's grain. However, there was a non significant trend for higher ratios of acetate to propionate for rations containing wet brewer's grain.

Palmquist and Conrad (1982) observed that the molar ratio of acetate : propionate was considerably increased when cows were fed with diets containing dried distiller's grains and solubles, reflecting a lower concentration of readily fermentable starch in the feedstuff. They further observed that the concentration of acetate, propionate and butyrate were 63.1, 23.6 and 13.5 molar per cent, respectively and A:P ratio was 2.68 in dairy cows fed with diets containing distiller's dried grains with solubles.

Varel and Kreikemeier (1994) observed acetate, propionate and butyrate concentration of 60.73, 14.86 and 8.07 mmol/l respectively and the A:P ratio of 4.09, when cows fed with brome grass supplemented with Amafern (*Aspergillus oryzae*).

Pre treatment of total mixed ration with exogenous fibrolytic enzymes (cellulase and xylanase) significantly improved the acetate, propionate and butyrate concentration in heifers (Hristov *et al.*, 1998 and Beauchemin *et al.*, 2000). Hristov *et al.* (1998) found acetate, propionate and butyrate production of 59.6, 31.9 and 13.6 mmol/l respectively and the A:P ratio of 2.07 in heifers fed with fibrolytic enzyme treated total mixed ration. Beauchemin *et al.* (2000) found acetate, propionate and butyrate concentration of 65.23, 21.17 and 12.0 mmol/l respectively and A:P ratio was 3.47 in cows when fed with enzyme treated total mixed ration (1.22 L of enzyme supplement per tonne) containing 40 per cent forage.

Similarly, Hristov *et al.* (2000) found significant improvement in the acetate, propionate and butyrate concentration of total mixed ration when exogenous polysaccharide degrading enzymes were infused into the rumen of heifers. They further reported acetate, propionate and butyrate concentration of 53.1, 34.9 and 9.83 mmol/l, respectively and A:P ratio of 1.69 in heifers when exogenous polysaccharide degrading enzymes (200g/day) were infused into the rumen.

Nichols *et al.* (1998) found significant increase in acetate and reduction in the propionate concentration in cows fed with diets containing distiller's grain compared to those fed with soybean meal based diet which is in agreement with Schingoethe *et al.* (1999).

Peter *et al.* (2000) found acetate, propionate and butyrate concentration of 32.6, 14.2 and 6.8 mmol/l respectively in beef cattle heifer fed with diets containing corn dried distiller's grain, whereas, Al-suwaeigh *et al.* (2002) observed 53.9, 24.9, 16.38 mmol/l of acetate, propionate, butyrate, respectively in dairy cows fed with corn dried distiller's grains based diet. They further reported acetate, propionate and butyrate concentration of 54.4, 25.2, 15.5 mmol/l, respectively in dairy cows fed with diets containing sorghum dried distiller's grain. Anderson *et al.* (2006) found significant improvement in the butyrate concentration when lactating dairy cows were fed with diets containing dried corn distiller's grains compared to those fed with wet corn distiller's grains.

However, no significant difference was observed in acetate and propionate concentration and A:P ratio of total mixed ration as a result of distiller's grain addition in lactating dairy cows compared to those fed with corn and soyabean based control diet (Al-Suwaiegh *et al*., 2002 and Anderson *et al*., 2006).

Significant reduction in A:P ratio and increase in propionate concentration were observed in cows fed with total mixed ration as a result of yeast culture addition (Dawson *et al.*, 1990; Moloney and Drennan, 1994 and Plata *et al.*, 1994).

2.6.5 Gas Production

Addition of both wet and dry distiller's grain in total mixed ration significantly reduced the methane emission by 20 to 30 per cent in cows (Wood and Knipmeyer, 1998; Gizzi and Givens, 2001 and Holmberg, 2004). Smith (2005) observed fermented grain (brewer's grain and distiller's grain) feeding to ruminants, significantly reduced the methane emission by 20 to 50 per cent in emission factor (Methane/gross energy intake percentage).

2.6.5.1 Rusitec

Durand *et al.* (1988) observed a significant increase in the *in vitro* total gas production using RUSITEC when wheat straw was treated with ammonia (0.87 l/day) and sodium hydroxide (1.31 l/day) compared to those with untreated (0.86 l/day) wheat straw. Liu and Orskov (2000) found cellulase (16 units) treatment of steam pre-treated paddy straw significantly improved the 24 hours *in vitro* cumulative gas production (27.50 ml) and rate of gas production (4.45 per cent per hour).

However, no significant difference was observed in 24 hours *in vitro* total gas production of fescue hay based pelleted diet using RUSITEC, as a result of cationomycin (Ionophore) supplementation (Bogaert *et al.*, 1989). Wang *et al.* (1998) studied the *in vitro* total gas production (24 hours) of alfalafa hay and concentrate after adding *Yucca schidigera* extract using RUSITEC and found no significant difference.

2.6.6 Carbon dioxide (CO_2) and Methane (CH_4) Emission

2.6.6.1 Rusitec

Addition of cationomycin (Ionophore) to fescue hay based pelleted diet significantly reduced the CH_4 emissions from 29.1 to 16.9 mmol per day in RUSITEC (Bogaert *et al.*, 1989). They further observed significant difference between CO_2 vs CH_4 emissions of fescue hay based pelleted diet with cationomycin supplementation (83.60 vs 15.19 molar per cent) compared to those with non supplemented group (76.11 vs 22.85 molar per cent), respectively, 6 hours after feeding in RUSITEC. Carro *et al.* (1995) found significant reduction in the *in vitro* CH_4 emission of grass and corn silage based diet using RUSITEC, when nylon bags with pore size of 40 μm (5.61 mmol/day) was used, compared to those with 100 μm pore size (6.43 mmol/day). They further observed no significant difference in CO_2 emission of grass and corn silage based diet in RUSITEC when nylon bags with pore size of 100 and 40 μm were used. Dong *et al.* (1999) observed significant difference in CO_2 and CH_4 emission of pretreated orchard grass hay with fibrolytic enzymes (30000 nova cellulase and 10000 nova xylanase units per kg DM) using RUSITEC (6.84 and 1.95 mmol/d, respectively) compared to those with non treated orchard grass hay (3.38 and 1.22 mmol/d respectively). They further reported that enzyme treatment of orchard grass hay resulted in increased CH_4 production due to increased methanogenic bacterial population in RUSITEC. Hess *et al.* (2003) found significant difference in CH_4 emissions of barley straw based diet using RUSITEC as a result of defaunation (74.71 mmol/day) compared to faunated group (7.64 mmol/day).

Wang *et al.* (1998) studied the *in vitro* 24 hour CH_4 production of alfalafa hay and concentrate after adding *Yucca schidigera* extract using RUSITEC and found no significant difference. Hess *et al.* (2007) found no significant difference in the CH_4 emission of *Vigna unguiculata* mixed with a tropical grass *Brachiaria humidicola* or *Leucaena leucocephala* using RUSITEC.

2.7 EFFECT OF FEEDING BREWER'S GRAIN AND DISTILLER'S GRAIN IN ANIMALS (FEEDING TRIAL)

2.7.1 Body Weight

Crickenberger and Johnson (1982) found significant improvement in the final body weight and average daily gain (ADG) of heifers fed corn silage plus wet brewer's grain (33.8 per cent of the diet DM) compared to those with control (corn silage without brewer's grain) diet at the end of the ll2 day feeding trial. Similarly, Trach (2003a) observed a significant improvement in the ADG of growing beef bulls fed with untreated and treated (3 per cent lime + 2 per cent urea) rice straw with wet brewer's grain supplementation (302 vs 107 and 476 vs 154 g/head/day, respectively) when compared to those fed with non supplemented untreated and treated rice straw. He further reported that ADG of growing beef bulls was significantly improved when fed with untreated and treated rice straw (3 per cent lime + 2 per cent urea) based diet containing 10 per cent whole cotton seed and 10 per cent green grass with supplementation of wet brewer's grain (419 vs 146 and 557 vs 339 g/head/day, respectively) compared to those fed with non supplemented untreated and treated rice straw. However, no significant difference was observed in the live weight changes of the cows as a result of wet brewer's grain supplementation (Chioua *et al.*, 1998).

2.7.2 Dry Matter Intake (DMI)

Palmquist and Conrad (1982) observed DMI of 13.0 and 15.5 kg/day respectively in Jersey and Holstein cows fed with distillers dried grain with solubles. Trach (2003a) observed a significant improvement in organic matter intake (OMI) of rice straw with wet brewer's grain supplementation (1.98 per cent live weight) over non-supplemented control group (1.84 per cent live weight) of growing beef bulls in feeding trial 1, whereas in feeding trial 2, he did not observe any significant difference in DMI of growing beef bulls fed with diets containing rice straw supplemented with wet brewer's grain.

Murdock *et al.* (1981) found no significant difference in DMI of cows fed with wet brewer's grain at 15 and 30 per cent of the dietary DM replacing soybean meal and barley in alfalfa hay and corn silage based diet. Similarly, no significant difference was observed in DMI of heifers and cows fed with diets containing 15 to 30 per cent of the dietary DM by wet brewer's grain (Crickenberger and Johnson, 1982; Hoffman and Armentano, 1988; West *et al.*, 1994; Belibasakis and Tsirgogianni, 1996 and Dhiman *et al*., 2003). No significant difference was also observed in DMI of cows fed with diets containing (10 to 20 per cent DM) wet or dried distiller's grain with solubles (Nichols *et al*., 1998; Liu *et al.*, 2000; Leonardi *et al*., 2005 and Anderson *et al*., 2006).

However, Davis *et al.* (1983) observed that the DMI of lactating dairy cows fed with diets containing pressed brewer's grain at 20, 30 and 40 per cent inclusion was significantly reduced (18.2, 17.1 and 14.8 kg/day, respectively), compared to those with corn silage based control diet (19.7 kg/day). Similarly, significant reduction was observed in DMI of cows fed with diets containing (20 to 30 per cent DM) distiller's grain (Palmquist and Conrad, 1982; Lahr *et al*., 1983; Schingoethe *et al*., 1999 and Hippen *et al*., 2003).

Kalbande (1995) found that DMI of cows increased with increasing UDP levels in concentrate mixtures containing RDP to UDP ratio of 65:35, 55:45 and 40:60 and the values were 3.14, 3.38 and 3.70 per cent of body weight, respectively.

Ally (2003) reported that average daily DMI was reduced from 3.49 to 2.97 per cent of body weight in cows when the undegradable protein levels was increased from 26.8 to 42.9 per cent, respectively.

Augustine (2008) studied the average daily DMI of cows fed with rations containing maize waste, tapioca starch waste, brewery waste and paddy straw after adding 100 g protected fat and found no significant difference due to protected fat supplementation.

2.7.3 Digestibility

2.7.3.1 Dry Matter

The dry matter digestibility of diets containing wet corn distiller's grain was 65 to 67 per cent as reported by Firkins *et al.* (1985) and Birkelo *et al.* (2004) which were higher than that of dried corn distiller's grain and dried sorghum distiller's grain incorporated diets (60 to 62 per cent, respectively), as reported by Peter *et al.* (2000) and Harborth *et al.* (2006) in ruminants. Similarly, organic matter digestibility of diets containing wet corn distiller's grain and wet sorghum distiller's grain was 80 to 85 per cent as reported by Ham *et al.* (1994), Lodge *et al.* (1996), Lodge *et al.* (1997b) and that of dried corn distiller's grain and dried sorghum distiller's grain incorporated diet was 72 to 74 per cent as reported by Lodge *et al.* (1996) and Lodge *et al.* (1997a).

Firkins *et al.* (1985) found higher DM digestibility (67 per cent) of diets containing dried corn distiller's grain in ruminants, whereas, Peter *et al.* (2000) found lower organic matter digestibility of 65 per cent in diets containing dried corn distiller's grain in lactating dairy cows.

Trach (2003a) observed a significant improvement in the organic matter digestibility of untreated and treated rice straw (3 per cent lime + 2 per cent urea) as a result of wet brewer's grain supplementation (59.0 vs 62.5 per cent and 62.5 vs 65.1 per cent, respectively) compared to those with non supplemented group in growing beef bulls.

Ally (2003) reported that cows fed with rations containing undegradable protein levels of 26.8 and 42.9 per cent had DM digestibility of 62.34 and 66.67 per cent, respectively.

Augustine (2008) found that cows fed with rations containing tapioca starch waste, brewery waste and paddy straw with and without addition of 100 g protected fat had DM digestibility of 56.48 and 50.99 per cent and found no significant difference due to protected fat supplementation.

2.7.3.2 Crude Protein

The crude protein digestibility of diets containing wet corn distiller's grain was 74 to 80 per cent as reported by Lodge *et al.* (1996) and Birkelo *et al.* (2004), while that of dried corn distiller's grain incorporated diet was 60 to 65 per cent as reported by Lodge *et al.* (1996) and Fastinger and Mahan (2006). Similarly, CP digestibility of diets containing both wet and dried sorghum distiller's grain was 74 to 77 per cent in lactating dairy cows as reported by Lodge *et al.* (1996) and Lodge *et al.* (1997a). Peter *et al.* (2000) found lower CP digestibility of 41 per cent for diets containing dried corn distiller's grain in lactating dairy cows.

Rao *et al.* (2001) found significant improvement in CP digestibility of diets containing yeasac (yeast culture) in rams. Ally (2003) reported that rations containing undegradable protein levels of 26.8 and 42.9 per cent had CP digestibility of 74.22 and 73.69 per cent, respectively. Augustine (2008) found that rations containing tapioca starch waste, brewery waste and paddy straw with and without addition of 100g protected fat had CP digestibility of 65.75 and 62.25 per cent, respectively and found no significant difference due to protected fat supplementation.

2.7.3.3 Crude Fibre

Peter *et al.* (2000) found crude fibre digestibility of diets containing dried corn distiller's grain was 42 per cent in lactating dairy cows. Rao *et al.* (2001) found significant improvement in the crude fibre digestibility of diets containing yeasac (yeast culture) in rams.

The NDF digestibility of diets containing both wet and dried distiller's grain was 60 to 70 per cent in ruminants as reported by Firkins *et al.* (1985), Ham *et al.* (1994), Al-Suwaiegh *et al.* (2002) and Birkelo *et al.* (2004). The higher NDF digestibility of diets containing distiller's grain was 76 per cent in lactating dairy cows as reported by Lodge *et al.* (1996) and Lodge *et al.* (1997b). Plata *et al.* (1994) found significant improvement in NDF digestibility of low quality forages as a result of *Saccromyces cerevisiae* addition.

Ally (2003) reported that crude fibre digestibility of 52.07 and 55.67 per cent in cows for rations containing undegradable protein levels of 26.8 and 42.9 per cent, respectively. Augustine (2008) found that crude fibre digestibility in cows fed with rations containing tapioca starch waste, brewery waste and paddy straw with and without addition of 100 g protected fat was 38.08 and 44.13 per cent, respectively and found no significant difference due to protected fat supplementation.

The ADF digestibility of diets containing both wet and dried distiller's grain was 55 to 65 per cent as reported by Al-Suwaiegh *et al.* (2002) and Birkelo *et al*. (2004). Firkins *et al.* (1985) found higher ADF digestibility (72 per cent) with diets containing dried corn distiller's grain in ruminants.

2.7.4 Milk Yield

Conrad and Rogers (1977) found significant improvement in the milk production when cows fed with rations containing wet brewer's grain (20 per cent DM) compared to those fed with dried brewer's grain. Similarly, inclusion of wet brewer's grain (20 to 30 per cent DM) to the ground maize and maize silage based diets significantly improved the milk production in lactating dairy cows (Polan *et al*., 1985; Hoffman and Armentano, 1988 and Belibasakis and Tsirgogianni, 1996).

Palmquist and Conrad (1982) observed milk production of 20.3 and 28.8 kg/day respectively in Jersey and Holstein cows fed with diets containing distillers dried grain with solubles. Similarly, inclusion of both wet and dried distiller's grain to corn silage and alfalfa based diets significantly improved the milk production in lactating dairy cows (Owen and Larson, 1991; Powers *et al*., 1995; Nichols *et al*., 1998 and Anderson *et al*., 2006).

However, Davis *et al.* (1983) found significant reduction in the milk production of lactating dairy cows fed with diets containing 20, 30 and 40 per cent pressed brewer's grain (25.0, 24.4 and 22.2 kg per day, respectively) compared to those fed with soybean meal based (control) diet (25.6 kg per day). Chioua *et al.* (1998) also observed significant reduction in milk production of dairy cows fed with diets containing wet brewer's grain. Similarly, Hippen *et al.* (2003) found significant reduction in milk production of dairy cows fed with total mixed ration containing wet distiller's grain (30 per cent DM).

However, no significant difference was observed in the milk production of lactating dairy cows fed with diets containing 15 to 30 per cent of wet brewer's grain on DM basis (Murdock *et al*., 1981 ; Hoffman and Armentano, 1988 ; West *et al*., 1994 and Miyazawa *et al.*, 2007). Similarly, no significant difference was observed in the milk production of dairy cows fed with diets containing (15 to 20 per cent DM) both wet and dried distiller's grain (Al-Suwaiegh *et al*., 2002; Dhiman *et al*., 2003; Hippen *et al*., 2003 and Kalscheur *et al*., 2004).

Kalbande (1995) observed significant difference in average daily milk production of cows (10.11, 7.18 and 6.32 kg) fed on three concentrate mixtures containing RDP to UDP ratio of 40:60, 55:45 and 65:35, respectively. Ally (2003) reported that cows fed with rations containing undegradable protein levels of 26.8 and 42.9 per cent had average daily milk production of 9.08 and 8.23 kg, respectively.

2.7.5 Fat Corrected Milk (FCM)

Palmquist and Conrad (1982) observed four per cent fat corrected milk (FCM) yield of 22.9 and 27.4 kg/day, respectively, in Jersey and Holstein cows fed with diets containing distillers dried grain with solubles. They further observed no significant difference in milk fat yield between two breeds but significant reduction was observed in milk protein yield in Jersey cows fed with diets containing distiller's dried grain with solubles.

Belibasakis and Tsirgogianni (1996) and Chioua *et al.* (1998) observed significant improvement in the four per cent FCM yield of lactating dairy cows fed with diets containing wet brewer's grain (25.1 kg per day) when compared to those with maize silage and ground maize based control diet (21.1 kg per day). However, no significant difference was observed in the four per cent FCM yield of lactating dairy cows fed with diets containing (15 to 30 per cent DM) both wet and dried brewer's grains (Hoffman and Armentano, 1988 and Dhiman *et al*., 2003).

Augustine (2008) studied the four per cent FCM yield of cows fed with rations containing maize waste, tapioca starch waste, brewery waste and paddy straw after adding 100 g protected fat and found no significant difference due to protected fat supplementation.

2.7.6 Milk Composition

Hoffman and Armentano (1988) found no significant difference in milk composition of cows fed with diets containing (20 to 25 per cent DM) both wet and dried brewer's grains. Similarly, no significant difference was observed in milk composition such as milk protein, lactose, solids not fat and somatic cell counts of milk of lactating dairy cows fed with diets containing wet brewer grains (Chioua *et al.*, 1998 and Miyazawa *et al.*, 2007).

However, Anderson *et al*. (2006) found significant improvement in the milk fat, milk protein and milk urea nitrogen (MUN) concentration of dairy cows fed with diets containing (10 to 20 per cent DM) both wet and dried distiller's grains.

Augustine (2008) studied the milk composition such as milk total solids, milk fat, solids not fat, milk protein and MUN of cows fed with rations containing maize waste, tapioca starch waste, brewery waste and paddy straw after adding 100 g protected fat and found no significant difference due to protected fat supplementation.

2.7.6.1 Milk Fat

Palmquist and Conrad (1982) observed milk fat concentration of 4.80 and 3.69 per cent, respectively in Jersey and Holstein cows fed with diets containing distillers dried grain with solubles. Anderson *et al.* (2006) found significant improvement in the milk fat concentration of dairy cows when fed with alfalfa based diets containing (20 per cent DM) wet distillers grains with solubles (3.55 per cent) compared to those with dried distillers grains with solubles (3.28 per cent).

Similarly, Davis *et al.* (1983) found significant improvement in the milk fat concentration of lactating dairy cows fed with diets containing 20, 30 and 40 per cent pressed brewer's grain (4.2, 4.0 and 4.4 per cent, respectively) compared to those fed with soybean meal based (control) diet (3.6 per cent). Belibasakis and Tsirgogianni (1996) observed significant improvement in the milk fat concentration of dairy cows fed with diets containing wet brewer's grain (4.08 per cent) compared to those fed with maize silage, ground maize based control diet (3.82 per cent).

However, no significant difference was observed in the milk fat concentration of dairy cows fed with diets (15 to 30 per cent DM) containing both wet and dried brewer's grains (Murdock *et al.*, 1981; Seymour and Polan, 1986; Johnson *et al.*, 1987; Chioua *et al.*, 1998; Hoffman and Armentano, 1988 and Dhiman *et al*., 2003). Similarly, Al-Suwaiegh *et al.* (2002) and Anderson *et al.* (2006) found no significant difference in milk fat concentration of dairy cows which were fed with alfalfa based diets containing dried distiller's grains with solubles (20 to 25 per cent DM).

2.7.6.2 Total Solids

Johnson *et al.* (1987) found no significant difference in milk total solids content of dairy cows fed with diets containing wet brewer's grains (25 per cent DM) compared to those with soyabean meal based diet.

Belibasakis and Tsirgogianni (1996) observed significant improvement in the milk total solids content of dairy cows fed with diets containing wet brewer's grain (12.89 per cent) compared to maize silage, ground maize based control diet (12.44 per cent). However, Chioua *et al.* (1998) found significant reduction in the milk total solids content of Holstein cows fed with basal diet containing wet brewer's grain (10 per cent DM) and fresh bean curd pomace compared to soyabean meal based control diet.

2.7.6.3 Solid Not Fat (SNF)

Belibasakis and Tsirgogianni (1996) found no significant difference in solids not fat (SNF) content of milk of dairy cows fed with diets containing wet brewer's grain (8.81 per cent) compared to those fed maize silage, ground maize based control diet (8.62 per cent). Similarly, Dhiman *et al.* (2003) found no significant difference in solid not fat (SNF) content of dairy cows fed with diets containing (15 per cent DM) both wet (8.57 per cent) and dried brewer's grain (8.59 per cent).

2.7.6.4 Milk Protein

Anderson *et al.* (2006) found significant increase in milk protein content of dairy cows when fed with alfalfa based diets containing (10 per cent DM) wet distillers grains with solubles (3.11 per cent) compared to those with dried distillers grains with solubles (3.01 per cent).

Palmquist and Conrad (1982) observed feeding distillers dried grain with solubles based diet significantly reduced milk protein content in Jersey cows (2.85 per cent) compared to those with Holstein cows (3.40 per cent). Davis *et al.* (1983) found no significant difference in milk protein content of lactating dairy cows fed with diets containing 20, 30 and 40 per cent pressed brewer's grain (3.8, 3.7 and 3.6 per cent, respectively) compared to those fed with soyabean meal based (control) diet (3.8 per cent). Similarly, no significant difference in milk protein content could be observed in lactating dairy cows fed with diets containing (10 to 25 per cent DM) both wet and dried brewer's grains (Seymour and Polan, 1986 ; Johnson *et al*., 1987 ; Hoffman and Armentano, 1988 ; Belibasakis and Tsirgogianni, 1996 and Dhiman *et al*., 2003). However, West *et al*. (1994) and Chioua *et al.* (1998) observed significant reduction in milk protein content of lactating dairy cows fed with diets containing wet brewer's grains.

2.7.6.5 Milk Urea Nitrogen (MUN)

Bector *et al*. (1998) reported that MUN concentration in lactating dairy cows ranged from 22.8 to 92.4 mg per cent. Dhiman *et al.* (2003) found no significant difference in MUN concentration of lactating dairy cows fed with diets containing (15 per cent DM) both wet (15.38 mg/dl) and dried brewer's grain (15.88 mg/dl).

Anderson *et al.* (2006) found no significant difference in MUN concentration of dairy cows when fed with alfalfa based diets containing (20 per cent DM) wet distillers grains with solubles (14.09 mg/dl) compared to those fed with dried distillers grains with solubles (12.36 mg/dl).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

About six tonnes of brewery waste (fresh form) was collected for the experiment from Osho Farm, Pattikad, 12 kilometers away from the College of Veterinary and Animal Sciences, Mannuthy. About 500 grams each of wet brewery waste was collected at six different time periods to study the variability in chemical composition. Each sample was dried in a hot air oven at 60^0C for 48 hours and ground to pass through 1mm sieve. The ground samples were preserved in air tight containers for further chemical analysis.

Two experiments were conducted, one to assess the effect of brewery waste on nutrient digestibility of paddy straw based diet in lactating cows and the other to study the *in vitro* disappearance, degradability and rumen fermentation characteristics of various experimental feeds in Rumen Simulation Technique (RUSITEC).

3.1 *IN VIVO* RUMEN DEGRADABILITY STUDIES IN LACTATING COWS

3.1.1 Experimental Animals

The investigation was carried out for a period of 60 days at University Livestock Farm and Fodder Research and Development Scheme (ULF and FRDS), Mannuthy using early lactating crossbred cows (within 20 days after calving) to assess the effect of brewery waste incorporated paddy straw based diet on production performance.

Eighteen crossbred cows having a peak yield of minimum eight litres in the previous lactation, were selected from the herd maintained at the ULF and FRDS, Mannuthy as experimental animals. The animals were divided into three groups of six each as uniformly as possible with regard to age, body weight, pairity, previous milk yield and stage of lactation and were randomly allotted to three dietary treatments T1, T2 and T3.

3.1.2 Housing and Management

All the experimental cows were housed in the same shed with facilities for individual feeding and watering. Stall feeding was practiced throughout the experimental period. The animals were washed every day in the morning before 9 A.M. Stalls were cleaned twice daily before the morning and afternoon milking with frequent removal of dung.

3.1.3 Experimental Ration

The animals were fed with iso caloric and iso nitrogenous experimental rations comprising of concentrate mixtures having 70 per cent TDN and 20 per cent CP on DM basis and paddy straw formed the sole roughage. The concentrate mixture and paddy straw were fed depending upon their body weight and level of production as per NRC (1989).

The three dietary treatments used in the feeding trial were

T1- Control concentrate mixture and paddy straw as roughage (Control ration)

T2- Experimental concentrate mixture containing brewery waste at 25% level (brewery waste fed separately) and paddy straw as roughage

T3- Experimental concentrate mixture and brewery waste (25% of experimental concentrate mixture) incorporated paddy straw as roughage.

The ingredient composition of the concentrate mixtures are represented in Table 1.

The brewery waste was incorporated in concentrate mixture at the level of 25 per cent (on DM basis) in treatment 2 (T2) and treatment 3 (T3). In T2, the brewery waste was fed separately on wet basis. While in T3, the brewery waste to be incorporated in the concentrate mixture was treated with required quantity of paddy straw for three days before feeding. An adaptation period of seven days was given to each animal.

3.1.4 Feeding

The experimental animals were stall fed with the three rations. The calculated nutrient content of the rations are given in Table 2. The experimental animals had free access to clean, wholesome drinking water. The animals were fed with the concentrate twice daily, in the morning and in the afternoon before milking. Paddy straw was fed in 3 divided lots every day to ensure minimum wastage. The balance of the concentrate and roughage left behind by each animal was collected and weighed separately every day to calculate the actual dry matter intake. Individual data on the daily DM intake and milk produced were recorded throughout the experiment. Body weight of animals was recorded in the beginning and at the end of the experiment.

3.1.5 Milk Samples

Milk samples were collected at fortnightly intervals and were analysed for total solids, fat (IS: 1224, 1977), protein (AOAC, 1990, N*6.38), solids not fat (SNF) and milk urea nitrogen (MUN) (Bector *et al.*1998). Data on milk production of individual animal was recorded daily throughout the experimental period.

3.1.6 Hematological Parameters

Blood samples (10 ml) were collected on 0, 30th and 60th day of the experiment in sterile tubes containing 0.2 ml of 1 per cent heparin as anticoagulant and the plasma was separated immediately. These samples were used to determine haemoglobin (cyanmethaemoglobin method), plasma inorganic phosphorus (phosphomolybdate method), plasma urea nitrogen (modified Berthelot method) and plasma glucose (GOD-PAP method) using the kits supplied by Agappe diagnostics, India.

3.1.7 Rumen Fermentation Parameters

Rumen liquor was collected from all the animals using a stomach tube at the end of the feeding trial and was analysed for pH (using pH meter, ELICO

India Ltd, Hyderabad), total volatile fatty acids (Barnett and Reid, 1957) and ruminal ammonia nitrogen (Beecher and Whitten, 1970).

3.1.8 Digestibility Trial

A digestibility trial was conducted during the last seven days of the experiment. Dung samples collected each day were weighed and samples at the rate of one per cent of the total weight were collected from each animal in double lined polythene bags after mixing thoroughly. The samples collected each day were kept frozen in a deep freezer. After the end of the trial, samples collected for each animal for the seven days were pooled, mixed together and sub samples were taken for analysis. The moisture was determined immediately and the crude protein was estimated using the fresh samples. The rest of the analysis was done in dried samples. During the collection period, feed samples were collected daily from each animal and at the end of the trial, feed samples collected each day for each animal were pooled and sub samples were taken for analysis.

3.1.8.1 Sampling of Feeds

Representative samples of both concentrate and roughage offered and the balance left behind were taken everyday during the digestion trial for proximate analysis. Dry matter content of the feed was determined everyday and the composite samples were taken after pooling the samples collected on all the seven days of the trial.

3.1.8.2 Analysis of Feed and Dung

Feed samples (concentrate mixture, brewery waste and paddy straw) and dung samples were analyzed for dry matter (DM), organic Matter(OM), crude protein (CP), ether extract (EE), crude fibre (CF), total ash, acid insoluble ash (A.O.A.C., 1990). Fibre fractions such as NDF, ADF, hemicellulose, cellulose and lignin were analyzed by the method suggested by A.O.A.C. (1990). The calcium content of the samples was estimated by Atomic Absorption

Spectrophotometer using hollow cathode tube and phosphorus content by Vanado-Molybdate method (AOAC, 1990).

Data obtained from the digestion trial were used for the calculation of digestibility of DM, CP, EE, NFE, NDF and ADF.

3.2 *IN VITRO* RUMEN DEGRADABILITY STUDIES USING RUMEN SIMULATION TECHNIQUE (RUSITEC)

The *in vitro* disappearance of dry matter, organic matter, crude protein, crude fibre and fibre fractions such as neutral detergent fibre (NDF), acid detergent fibre (ADF), hemicellulose and cellulose of experimental feeds such as control concentrate mixture, paddy straw, brewery waste, experimental concentrate mixture incorporated with fresh brewery waste, experimental concentrate mixture incorporated with dried brewery waste and paddy straw treated with brewery waste for 3 days was determined using the rumen simulation technique (RUSITEC) described by Czerkawski and Breckenridge (1977).

The following seven treatments were incubated in the eight reaction vessels of the RUSITEC.

1. Control feed (CF)
2. Experimental feed (75%) + Fresh brewery waste (25%) (EFFBW)
3. Experimental feed (75%) + Dried brewery waste (25%) (EFDBW)
4. Fresh brewery waste (FBW)
5. Dried brewery waste (DBW)
6. Paddy straw (PS) and
7. Brewery waste incorporated paddy straw (BWIPS)

The experiment was replicated.

Proximate analysis and fibre fractions of above mentioned treatments were carried out as per standard procedure (AOAC, 1990).

The fermentation parameters viz., pH, total gas production, CO_2 production, total volatile fatty acids (TVFA) and their fractions, ammonia nitrogen, disappearance of dry matter, crude protein, crude fibre and fibre fractions were studied in RUSITEC.

The detailed experimental procedures are as follows:

Rumen digesta and rumen liquor were collected from six cows immediately after slaughter from the District Slaughter House, Kuriachira, Thrissur. It was thoroughly mixed and transported to the laboratory (within 30 minutes) in a pre-heated vacuum flask (39^{o}C). Handling of rumen liquor in the laboratory was carried out by continuous flushing with CO_2. The rumen fluid was strained through a double-layered muslin cloth into a CO_2 filled beaker.

Each reaction vessel was charged with 500 ml of strained rumen liquor and 200 ml of artificial saliva (McDougall, 1948). One nylon bag (pore size 100 μm) containing 80g of rumen solid digesta (fibrous residue from the rumen content straining) and another containing 10 g (dry matter) of feed to be tested were placed into the perforated feed container and the assembly was put into the reaction vessel which was filled up to the brim with distilled water making the total volume of the container to one liter.

The composition of one litre artificial saliva is as follows:

Sodium hydrogen carbonate (NaHCO3)	—	9.80 g
Disodium hydrogen ortho phosphate ($Na_2HPO_4 . 2H_2O$)	—	7.00 g
Potassium chloride (KCl)	—	0.57 g
Sodium chloride (NaCl)	—	0.47 g
Magnesium sulphate ($MgSO_4 . 2H_2O$)	—	0.12 g
Calcium chloride ($CaCl_2$)	—	0.04 g

The artificial saliva was prepared with one litre distilled water and kept at 39^{o}C and then carbon dioxide was infused into it.

Artificial saliva was pumped at a constant ratio of infusion (650 ml/day) into the reaction vessel by a peristaltic pump. The effluent and fermentation gases were collected in effluent collection vessels (containing few drops of saturated $HgCl_2$ solution) and gas collection bags, respectively. After 24 hours the solid inoculum was removed and a new bag of feed was placed in the feed container. Thus each reaction vessel at a time contained 2 bags introduced each in 2 consecutive days and removed 48 hours later.

The bag to be removed was allowed to drain, squeezed and washed in artificial saliva in a polyethylene bag. The washings were returned to the respective reaction vessels. The removed bags were further washed and dried at 60^oC for 48 hours. Each experiment totally consisted of 7 days adaptation period followed by collection period.

3.2.1 *In Vitro* Rumen Fermentation Parameters in RUSITEC

The pH of effluent collected at various time intervals was determined using digital pH meter (ELICO India Ltd, Hyderabad). The ammonia nitrogen concentration of the effluents collected was estimated colorimetrically as per the method of Beecher and Whitten (1970). At the end of specific time intervals the infusion of artificial saliva was stopped and the gas collected in the gas bag was measured for the total gas production (by the concept of replacing the water by gas while pressing the gasbag attached to the 3litre capacity glass bottle with two-way valve) and fractioned to CO_2 and CH_4 using saturated potassium hydroxide.

For measuring CO_2 production, 2ml of gas from the reaction vessel was taken by inserting 22 gauge needle into the three way cannula dummy rubber cork attached to the reaction vessel. Immediately after collection, the gas was passed into another 5ml glass syringe (through rubber cork) containing 2ml of saturated potassium hydroxide. The CO_2 will be dissolved in saturated potassium hydroxide which can be measured.

3.2.2 *In Vitro* Dry Matter and Crude Protein Degradability Studies in RUSITEC

Loss in weight of nylon bag after 0, 2, 6, 12, 24, 48 and 72 hours of incubation in RUSITEC followed by washing and drying was recorded to calculate dry matter disappearance. The i*n vitro* disappearance of nutrients was calculated using the following formula and expressed as percentage on dry matter basis.

$$\textit{In vitro}\text{ disappearance} = \frac{\text{(weight of bag with sample before incubation)} - \text{(weight of bag with sample after incubation)}}{\text{weight of sample}} \times 100$$

The effective degradability of dry matter was calculated and results of the dry matter degraded at various time intervals and were fitted to exponential equation of Orskov and McDonald (1979) mentioned as below:

$P = a + b\,(1- e^{-ct})$

Where,
- P = Per cent of degradation at time t,
- a = Per cent soluble fraction.
- b = Insoluble but potentially degradable as percentage
- a+b = The value of potential degradability of the material as percentage
- c = The degradation rate, expressed as percentage/hour (a, b, c are constant in exponential equation).

The formula was modified to

$P = a + \{bc/(c+r)\}$ (Mc Donald *et al*. 2002).

Since, the fraction of nutrient remaining in the rumen as well as the rate of breakdown will be reduced to Zero as the time of incubation increases.

Where
- P= Per cent of potential degradation at time t,
- a = Per cent immediately degradable fraction
- b = Per cent slowly degradable fraction
- c = Rate of degradation of fraction b, expressed as percentage/hour
- r = Rate of passage which is 0.027 in RUSITEC.

(a, b, c are constant in exponential equation).

Similarly, crude protein degradability was measured in RUSITEC.

3.2.3 Volatile Fatty Acid (VFA) Fractions

3.2.3.1 Preparation of Sample

At the end of each incubation hour (2, 6, 12, 24, 48 and 72 hours), 1 ml sample was collected from reaction vessel and then added 5 ml metaphosphoric acid (25 per cent), kept the sample for 30 minutes and then centrifuged at 5000 rpm for 10 minutes. The clear supernatant was collected and used for individual short chain volatile fatty acid analysis.

3.2.3.2 VFA Analysis

Concentrations were measured by gas chromatograph method as per the procedure of Agarwal *et al.* (2008). Chemito make of gas chromatograph model "Chemito - CERES 800 PLUS" at Department of Veterinary Physiology, Veterinary College and Research Institute, Namakkal was used in this study. The concentrations of volatile fatty acids (mmol/L) were calibrated from the standards.

3.3 ECONOMICS

The cost of brewery waste, concentrate mixture and paddy straw for producing one kilogram of milk was calculated using the data on total feed consumption and total milk yield during the experimental period.

3.4 STATISTICAL ANALYSIS

The data on average daily milk yield was analysed statistically using Analysis of Covariance and rest of data were analysed using Analysis of variance (ANOVA) as per Snedecor and Cochran (1994). Means were compared by Duncan Multiple Range Test (DMRT) using SPSS package.

Table 1. Per cent ingredient composition of the control and experimental concentrate mixtures used

Ingredient	Concentrate mixture (%)	
	Control	Experimental
Yellow Maize	37.00	40.00
Groundnut Cake	29.00	21.50
Wheat bran	30.50	10.00
Brewery waste	0.00	25.00
Mineral Mixture*	1.00	1.00
Salt	1.00	1.00
Shell Grit	1.50	1.50

To every 100 kg of concentrate mixture 20 grams of Nicomix AB_2D_3K (Nicholas Piramal India Ltd, Mumbai) containing Vitamin A-82500 I.U, Vitamin D_3-12000 I.U, Vitamin B_2-50 mg, Vitamin K-10 mg per gram was added.

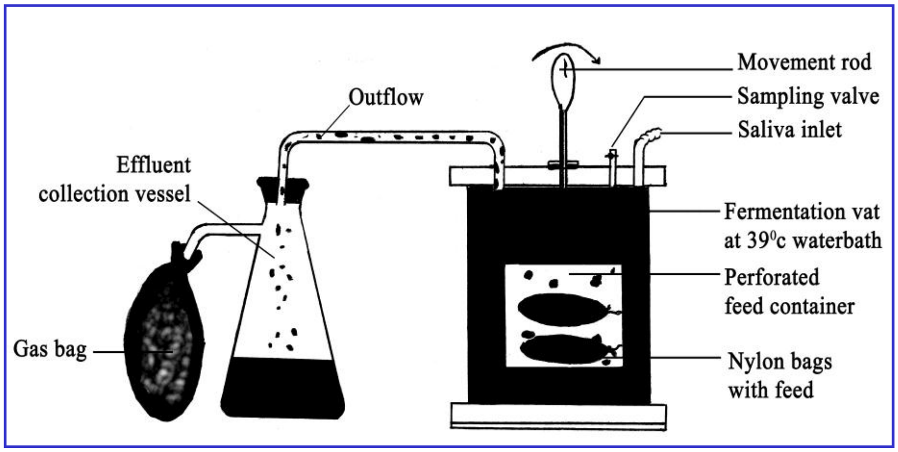
*Mineral mixture supplied by Kerala Feeds Ltd. Kerala, containing Calcium (minimum) 20 per cent, Phosphorus (minimum) 12 per cent, Magnesium (minimum) 5 per cent, Iron (minimum) 0.4 per cent, Copper (minimum) 0.1 per cent, Zinc (minimum) 0.8 per cent, Manganese (minimum) 0.12 per cent, Cobalt (minimum) 0.012 per cent, Iodine (minimum) 0.026 per cent, Sulphur 1.8 - 3 per cent, Arsenic (maximum) 7 ppm, Lead (maximum) 20 ppm and Flourine (maximum) 0.07 per cent.

Table 2. Calculated nutrient content of the control and experimental concentrate mixtures used

Nutrient	Concentrate mixture, %	
	Control	Experimental
TDN	70.02	70.02
CP	20.07	20.15
Ca	0.95	0.96
P	0.83	0.63

Plate 1. RUSITEC Apparatus

Plate 2. Reaction Vessel



RESULTS

4. RESULTS

The results obtained in the present study are given under the following headings.

4.1 CHEMICAL COMPOSITION

The data on the chemical composition, fibre fractions and mineral composition of the control and experimental concentrate mixtures, brewery waste, paddy straw and brewery waste incorporated paddy straw used for *in vivo* and *in vitro* (RUSITEC) experiments are given in Tables 3, 4 and 5, respectively.

The dry matter (DM) content of paddy straw, brewery waste and brewery waste incorporated paddy straw was 90.35 ± 0.81, 29.15 ± 0.43 and 78.11 ± 0.63 respectively. The crude protein (CP) content of brewery waste and brewery waste incorporated paddy straw was 24.34 ± 0.60 and 9.41 ± 0.44 per cent, respectively on DM basis. The CP content of control concentrate mixture and experimental concentrate mixture with brewery waste was 20.06 ± 1.05 and 20.12 ± 0.63 per cent, respectively.

The crude fibre (CF), total ash and nitrogen free extract (NFE) of brewery waste were 19.62 ± 0.31, 5.76 ± 0.14 and 45.07 ± 0.79 per cent, respectively, on DM basis. The gross energy (GE) content of brewery waste was 3543.52 ± 46.33 kcal/kg on DM basis.

The neutral detergent fibre (NDF), acid detergent fibre (ADF), hemicellulose, cellulose and lignin content of brewery waste were 54.64 ± 0.62, 24.68 ± 0.92, 29.96 ± 1.03, 13.14 ± 0.35 and 7.12 ± 0.26 per cent, respectively on DM basis.

The calcium and phosphorus content of brewery waste were 0.32 ± 0.03 and 0.60 ± 0.02 per cent, respectively on DM basis.

4.2 BODY WEIGHT

The mean body weight of experimental animals recorded at the beginning and end of feeding trial are documented in Table 6 and represented in Fig.1. The initial mean body weight of the animals in the three groups T1, T2, and T3 were 358.17 ± 21.15, 366.83 ± 15.37 and 336.00 ± 8.05 kg, respectively, while the final weight were 358.00 ± 20.78, 389.50 ± 16.57 and 354.50 ± 7.53 kg, respectively.

4.3 DRY MATTER INTAKE (DMI)

The weekly average of daily DMI of experimental animals maintained on three dietary treatment groups T1, T2 and T3 were 11.64 ± 0.26, 12.11 ± 0.45 and 12.10 ± 0.35 kg, respectively (Table 7 and Fig. 2).

The average DMI per 100 kg of body weight was 3.25, 3.11 and 3.41 kg, respectively, for the cows under the three treatments T1, T2 and T3. The average DMI per kg metabolic body weight was 14.14, 13.81 and 14.81 kg, respectively, for the cows maintained under the three treatments T1, T2 and T3 (Table 15).

4.4 MILK PRODUCTION

The mean weekly average of daily milk production of the experimental animals maintained on three dietary treatments T1, T2 and T3 was 8.03 ± 0.27, 10.14 ± 1.07 and 11.09 ± 0.56 kg, respectively (Table 8 and Fig. 3).

4.5 MILK COMPOSITION

Data on composition of milk viz. percentage of total solids, milk fat, solids not fat, milk protein and milk urea nitrogen (mg/100 ml) collected from experimental animals at fortnightly intervals are given in Table 9 and are graphically presented in Fig. 4, 5, 6, 7 and 8, respectively.

4.6 YIELD OF FOUR PER CENT FAT CORRECTED MILK (FCM), MILK FAT AND MILK PROTEIN

Data on the four per cent FCM yield, milk fat and milk protein yield during the entire period of experiment (60 days) is given in Table 10 and graphically presented in Fig. 9 and 10. Average four per cent FCM yield was 462.97 ± 20.85, 536.89 ± 57.08 and 533.68 ± 62.49 kg, respectively, for animals maintained on three dietary treatments T1, T2 and T3. The mean yield of milk fat was 17.36 ± 1.03, 20.01 ± 2.35 and 19.62 ± 2.30 kg, while the milk protein yield was 12.80 ± 0.74, 15.50 ±1.42 and 15.45 ± 1.87 kg, respectively, for the animals maintained on three dietary treatments T1, T2 and T3.

4.7 RUMEN FERMENTATION PARAMETERS

Data on the rumen fermentation parameters such as pH, ammonia nitrogen (NH_3-N) and total volatile fatty acids (TVFA) of the rumen liquor collected from the experimental animals at the end of the experiment are given in Table 11 and depicted graphically in Fig. 11, 12 and 13.

4.8 HAEMATOLOGICAL PARAMETERS

Data on haemoglobin, plasma glucose, plasma urea nitrogen, plasma calcium and phosphorus content of blood samples collected from the experimental animals at monthly intervals are presented in Table 12 and depicted graphically in Fig. 14.

4.9 APPARENT DIGESTIBILITY

The chemical composition of dung of the experimental animals maintained on three dietary treatments collected during digestibility trial is presented in Table 13.

The apparent digestibility (per cent) of the nutrients such as DM, OM, CP, CF, EE, NFE, gross energy and fibre fractions such as NDF, ADF, hemicellulose and cellulose of the three experimental rations T1, T2 and T3 is given in Table 14 and the data illustrated in Fig. 15 and 16.

4.10 ECONOMICS OF PRODUCTION

The total milk production and cost of feed per kg milk production of experimental cows maintained on three dietary treatments T1, T2 and T3 are depicted in Table 15. The cost of feed per kg milk production of experimental cows maintained on three dietary treatments T1, T2 and T3 are given in Fig. 17.

4.11 *IN VITRO* DISAPPEARANCE/DEGRADABILITY STUDIES IN RUSITEC

The percentage *in vitro* disappearance of DM, CP, CF, NDF, ADF, hemicellulose and cellulose of control feed, experimental feed with fresh brewery waste (EFFBW), experimental feed with dried brewery waste (EFDBW), fresh brewery waste (FBW), dried brewery waste (DBW), paddy straw (PS) and brewery waste incorporated paddy straw (BWIPS) incubated for 0, 2, 6, 12, 24, 48 and 72 hours in Rumen Simulation Technique (RUSITEC) are given in Tables 16, 17, 18, 19, 20, 21 and 22 and are illustrated in Fig. 18, 19, 20, 21, 22, 23 and 24, respectively.

Data on soluble fraction '*a*', degradable fraction '*b*', rate of degradation '*c*' and the percentage *in vitro* effective degradability of DM and CP of control feed, EFFBW, EFDBW, FBW, DBW, PS and BWIPS in RUSITEC are presented in Tables 23 and 24 and illustrated in Fig. 25 and 26, respectively.

4.12 *IN VITRO* RUMEN FERMENTATION CHARACTERISTICS

The *in vitro* total gas production (ml), carbon dioxide production (per cent), pH, NH_3-N (mg/100 ml), TVFA (mmol/l), and volatile fatty acid fractions (mmol/l) of control feed, EFFBW, EFDBW, FBW, DBW, PS and BWIPS incubated for 2, 6, 12, 24, 48 and 72 hours in RUSITEC are given in the Tables 25, 26, 27, 28, 29 and 30 and are illustrated in Fig. 27, 28, 29, 30, 31, 32, 33, 34, 35 and 36, respectively.

Table 3. Per cent chemical composition of the concentrate mixtures, brewery waste, paddy straw and brewery waste incorporated paddy straw* (on DM basis)

Nutrients	Concentrate mixture		Brewery waste	Paddy straw	Brewery waste incorporated paddy straw
	Control	Experimental			
Dry matter	95.15 ± 0.56	94.76 ± 0.26	29.15 ± 0.43	90.35 ± 0.81	78.11 ± 0.63
Crude protein	20.06 ± 1.05	20.12 ± 0.63	24.34 ± 0.60	4.42 ± 0.18	9.41 ± 0.44
Ether extract	4.84 ± 0.45	4.82 ± 0.28	5.19 ± 0.18	0.86 ± 0.05	1.94 ± 0.13
Crude fibre	6.34 ± 0.18	8.88 ± 0.35	19.62 ± 0.31	34.19 ± 0.61	30.55 ± 0.57
Total ash	8.32 ± 0.18	8.54 ± 0.16	5.76 ± 0.14	10.64 ± 0.25	9.42 ± 0.18
Nitrogen free extract	60.44 ± 0.87	57.64 ± 1.03	45.07 ± 0.79	49.89 ± 0.64	48.69 ± 0.61
Organic matter	91.68 ± 0.18	91.47 ± 0.16	94.25 ± 0.14	89.36 ± 0.25	90.58 ± 0.18
Acid insoluble ash	2.56 ± 0.21	2.68 ± 0.14	4.42 ± 0.15	8.76 ± 0.20	7.68 ± 0.18

* Mean of four values ± SE

Table 4. Per cent fibre fractions of concentrate mixtures, brewery waste, paddy straw and brewery waste incorporated paddy straw* (on DM basis)

Nutrients	Concentrate mixture		Brewery waste	Paddy straw	Brewery waste incorporated paddy straw
	Control	Experimental			
Neutral detergent fibre	15.24 ± 0.35	24.66 ± 0.37	54.64 ± 0.62	73.16 ± 1.33	68.53 ± 0.80
Acid detergent fibre	10.52 ± 0.23	13.68 ± 0.57	24.68 ± 0.92	48.57 ± 0.87	42.60 ± 0.84
Hemicellulose	4.72 ± 0.37	10.98 ± 0.55	29.96 ± 1.03	24.59 ± 0.50	25.93 ± 0.56
Cellulose	4.22 ± 0.24	6.46 ± 0.28	13.14 ± 0.35	34.56 ± 0.52	29.21 ± 0.56
Lignin	3.74 ± 0.27	4.54 ± 0.27	7.12 ± 0.26	5.25 ± 0.27	5.72 ± 0.28

* Mean of four values ± SE

Table 5. Mineral composition of paddy straw, brewery waste and brewery waste incorporated paddy straw* (on DM basis)

Item	Paddy straw	Brewery waste	Brewery waste incorporated paddy straw
Calcium (g/100 g)	0.28 ± 0.02	0.32 ± 0.03	0.28 ± 0.02
Phosphorus (g/100 g)	0.15 ± 0.02	0.60 ± 0.02	0.29 ± 0.02
Magnesium (g/100 g)	0.14 ± 0.01	0.16 ± 0.02	0.10 ± 0.01
Copper (ppm)	9.89 ± 0.98	14.00 ± 1.24	12.54 ± 1.16
Zinc (ppm)	8.21 ± 3.30	35.00 ± 0.02	15.18 ± 2.24
Iron (ppm)	230.17 ± 10.78	112.00 ± 7.85	174.36 ± 8.46
Manganese (ppm)	88.65 ± 6.71	21.00 ± 1.82	95.44 ± 5.78

* Mean of four values ± SE

Table 6. Body weight of experimental cows maintained on three dietary treatments*

Period, d	Body weight, kg		
	T1	T2	T3
0	358.17 ± 21.15	366.83 ± 15.37	336.00 ± 8.05
60	358.00 ± 20.78	389.50 ± 16.57	354.50 ± 7.53

*Mean of six values ± SE

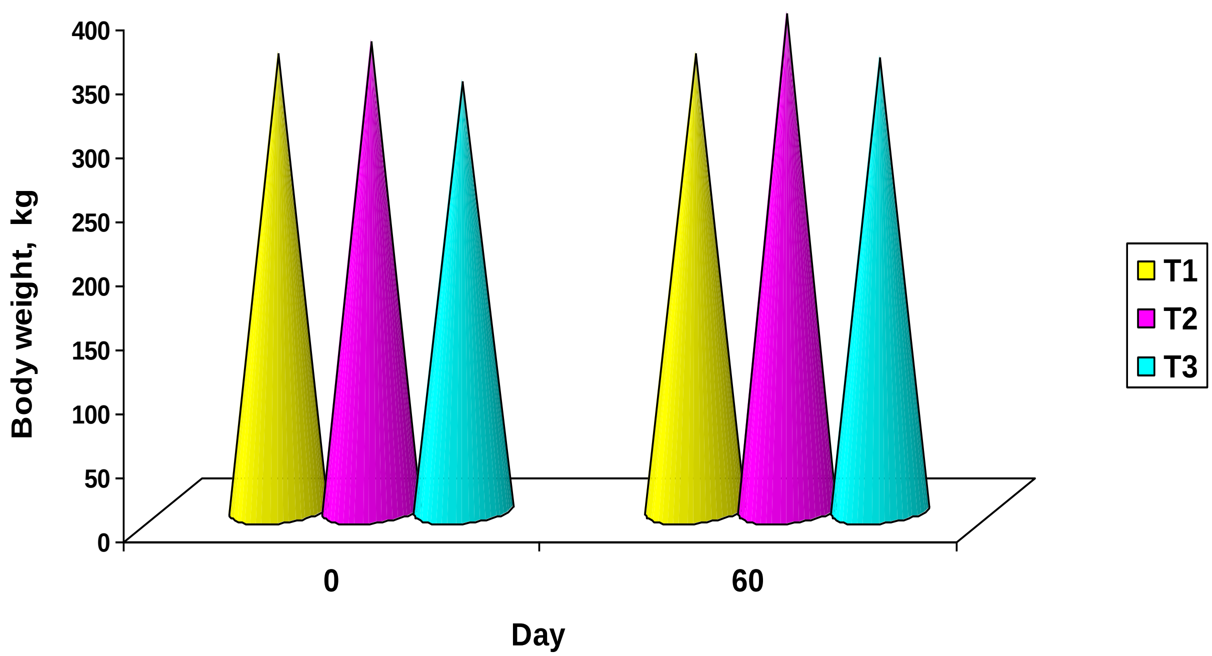


Fig. 1. Body weight of experimental cows maintained on three dietary treatments, kg

Table 7. Weekly average of daily dry matter intake of experimental cows maintained on three dietary treatments*

Week	Daily dry matter intake, kg		
	T1	T2	T3
1	11.29 ± 0.28	12.42 ± 0.63	11.97 ± 0.36
2	11.22 ± 0.12	12.07 ± 0.43	11.93 ± 0.37
3	11.53 ± 031	12.12 ± 0.45	12.07 ± 0.33
4	11.61 ± 0.29	12.12 ± 0.46	12.17 ± 0.32
5	11.78 ± 0.31	12.09 ± 0.45	12.18 ± 0.37
6	11.76 ± 0.29	12.14 ± 0.46	12.16 ± 0.34
7	11.89 ± 0.28	12.14 ± 0.47	12.16 ± 0.38
8	11.92 ± 0.27	12.19 ± 0.45	12.17 ± 0.37
Overall Mean ± SE	11.64 ± 0.26	12.11 ± 0.45	12.10 ± 0.35

*Mean of six values ± SE

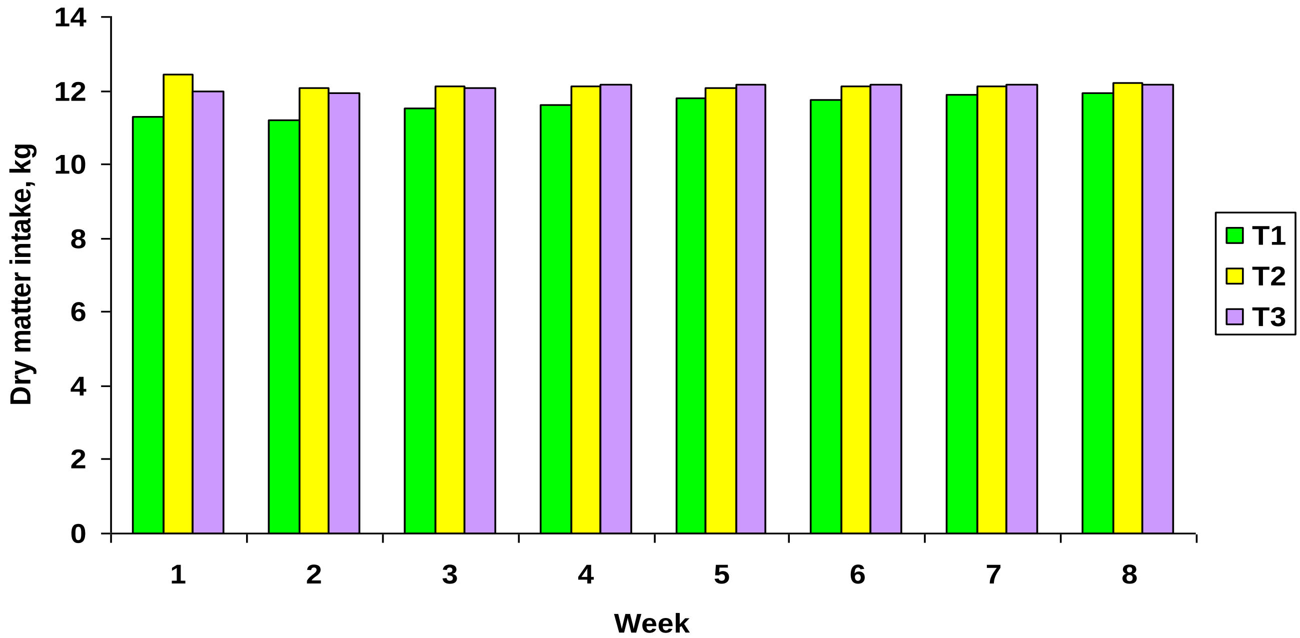


Fig. 2. Weekly average of daily dry matter intake of experimental cows maintained on three dietary treatments, kg

Table 8. Weekly average of daily milk production of experimental cows maintained on three dietary treatments*

Week	Daily milk production, kg		
	T1	T2	T3
1	8.62 ± 0.43	10.25 ± 1.28	11.28 ± 0.51
2**	8.37^{a} ± 0.35	10.39^{ab} ± 1.20	11.35^{b} ± 0.48
3***	8.35^{a} ± 0.43	10.43^{ab} ± 1.31	11.48^{b} ± 0.49
4	7.86^{a} ± 0.37	10.29^{b} ± 1.07	11.25^{b} ± 0.61
5	7.81^{a} ± 0.28	9.58^{ab} ± 0.77	10.74^{b} ± 0.74
6	7.76^{a} ± 0.17	9.77^{b} ± 0.93	10.54^{b} ± 0.40
7	7.97^{a} ± 0.23	9.95^{ab} ± 1.10	11.23^{b} ± 0.69
8	7.67^{a} ± 0.32	10.37^{b} ± 1.18	10.93^{b} ± 0.71
Overall Mean ± SE	8.03^{a} ± 0.27	10.14^{ab} ± 1.07	11.09^{b} ± 0.56

* Mean of six values ± SE

a,b – Means with different superscript within the same row differ significantly ($P<0.05$)

** P = 0.056 ; *** P = 0.071

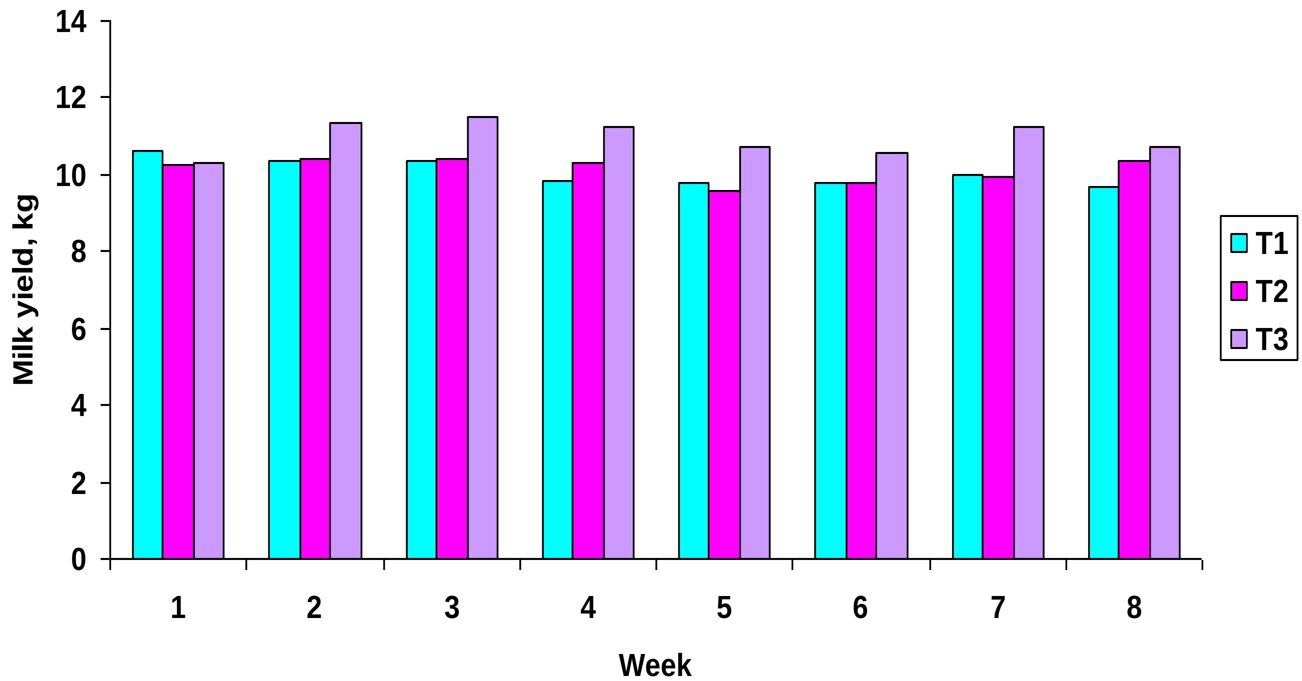


Fig. 3. Weekly average of daily milk production of experimental cows maintained on three dietary treatments, kg

Table 9. Composition of milk of experimental cows maintained on three dietary treatments*

Item	Treatment	Period, d				
		Initial	15	30	45	60
Total solids, %	T1	13.06 ± 0.25	12.55 ± 0.37	10.92 ± 0.45	12.06 ± 0.25	12.13 ± 0.29
	T2	12.27 ± 0.38	11.74 ± 0.45	11.24 ± 0.55	11.32 ± 0.32	12.27 ± 0.42
	T3	12.57 ± 0.37	11.47 ± 0.35	10.72 ± 0.20	11.48 ± 0.22	11.43 ± 0.12
Milk fat, %	T1	3.62 ± 0.12	3.43 ± 0.11	3.01 ± 0.25	3.70 ± 019	3.36 ± 0.19
	T2	3.55 ± 0.11	3.08 ± 0.26	3.27 ± 0.31	3.27 ± 0.12	3.58 ± 0.24
	T3	3.66 ± 0..27	3.41 ± 0.17	2.96 ± 0.24	3.18 ± 0.18	3.22 ± 0.16
Solids not fat, %	T1	9.44 ± 0.17	9.12 ± 0.42	7.92 ± 0.37	8.36 ± 0.10	8.77 ± 0.24
	T2	8.72 ± 0.28	8.65 ± 0.33	7.97 ± 0.38	8.05 ± 0.26	8.69 ± 0.32
	T3	8.92 ± 0.20	8.06 ± 0.39	7.76 ± 0.29	8.30 ± 0.21	8.21 ± 0.23
Milk protein, %	T1	2.75 ± 0.28	2.15 ± 0.08	2.14 ± 0.14	2.73 ± 0.09	2.86^{a} ± 0.06
	T2	2.53 ± 0.22	2.39 ± 0.06	2.33 ± 0.09	2.81 ± 0.08	3.07^{b} ± 0.07
	T3	2.82 ± 0.10	2.24 ± 0.07	2.40 ± 0.12	2.70 ± 0.04	2.76^{a} ± 0.07
Milk urea nitrogen, mg/100 ml	T1	47.20 ± 1.82	40.32^{a} ± 0.76	37.36 ± 2.04	40.04 ± 2.70	33.66^{a} ± 3.11
	T2	46.86 ± 4.00	40.70^{a} ± 1.72	37.95 ± 1.76	43.95 ± 2.38	45.87^{b} ± 0.76
	T3	40.22 ± 2.91	32.64^{b} ± 2.14	35.09 ± 3.17	35.42 ± 2.39	35.87^{a} ± 2.95

* Mean of six values ± SE

a,b – Means with different superscripts within the same column for each parameter differ significantly (P<0.05)

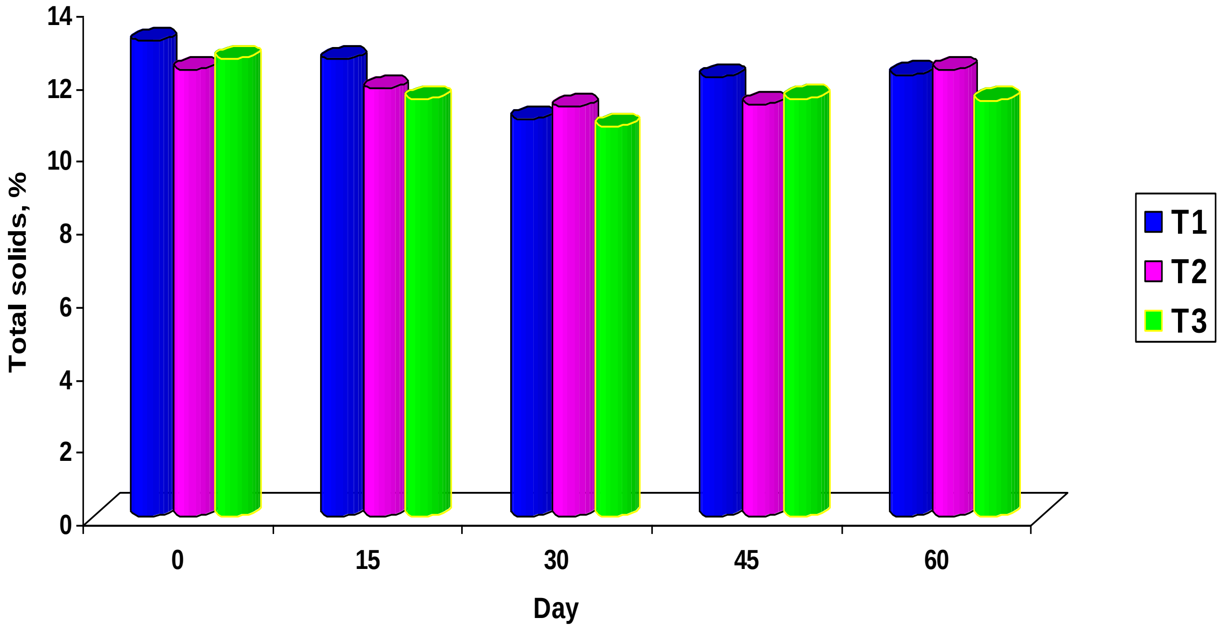


Fig. 4. Average milk total solids of experimental cows maintained on three dietary treatments

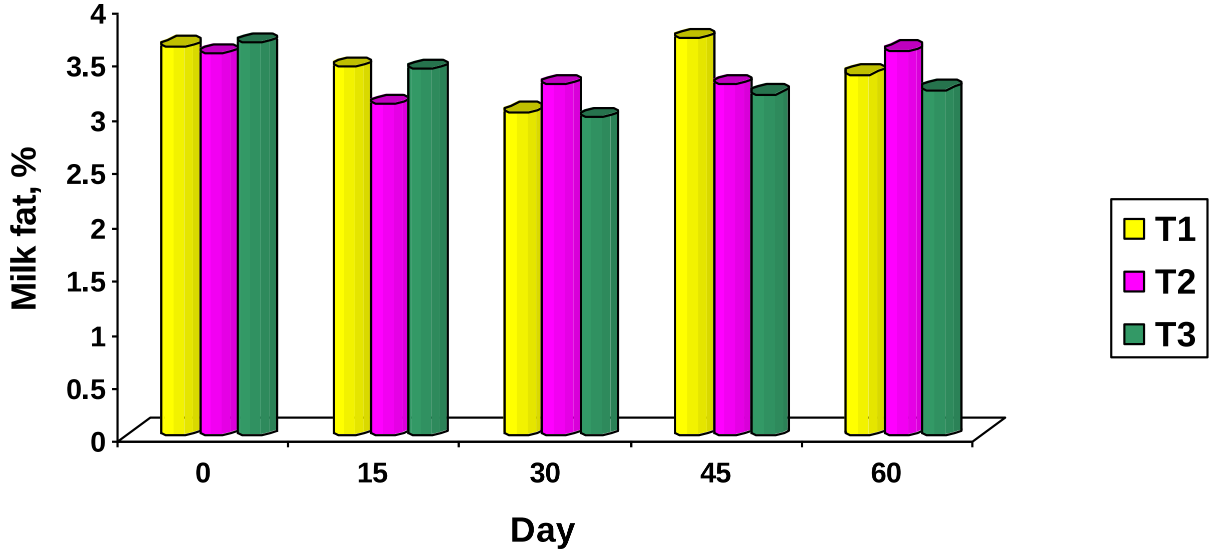


Fig. 5. Average milk fat of experimental cows maintained on three dietary treatments

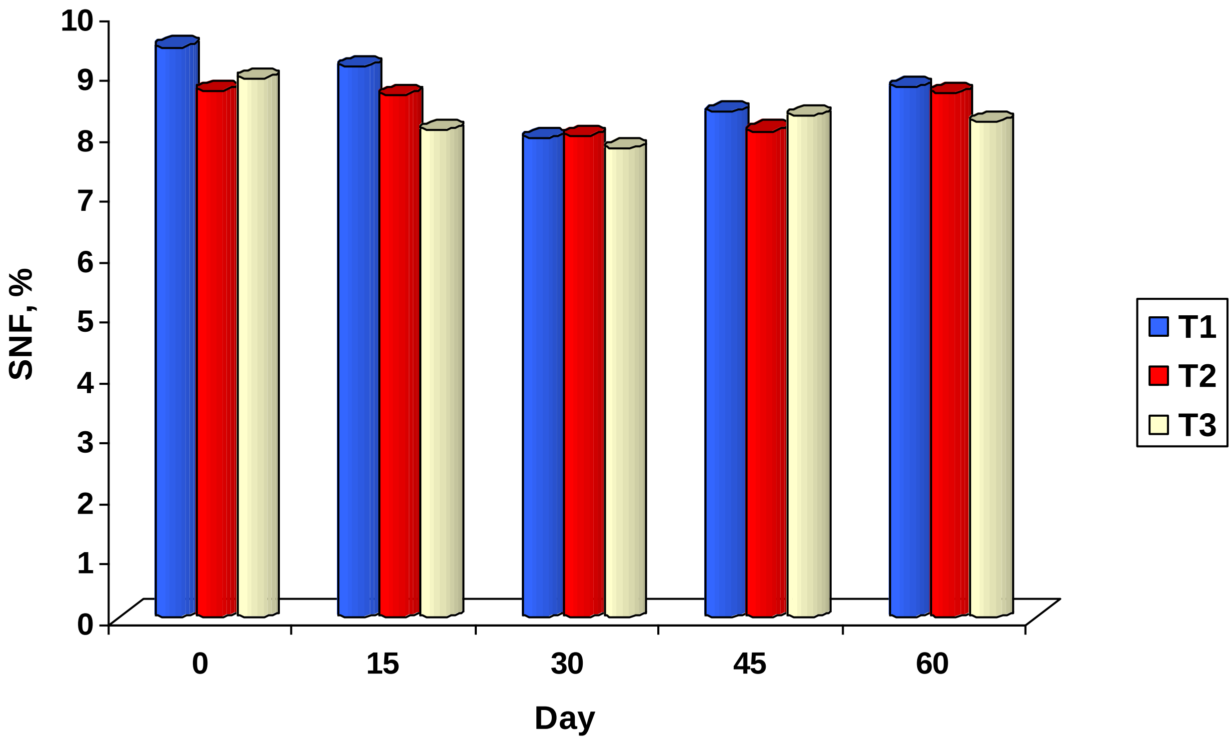


Fig. 6. Average solids not fat (SNF) content of milk of experimental cows maintained on three dietary treatments

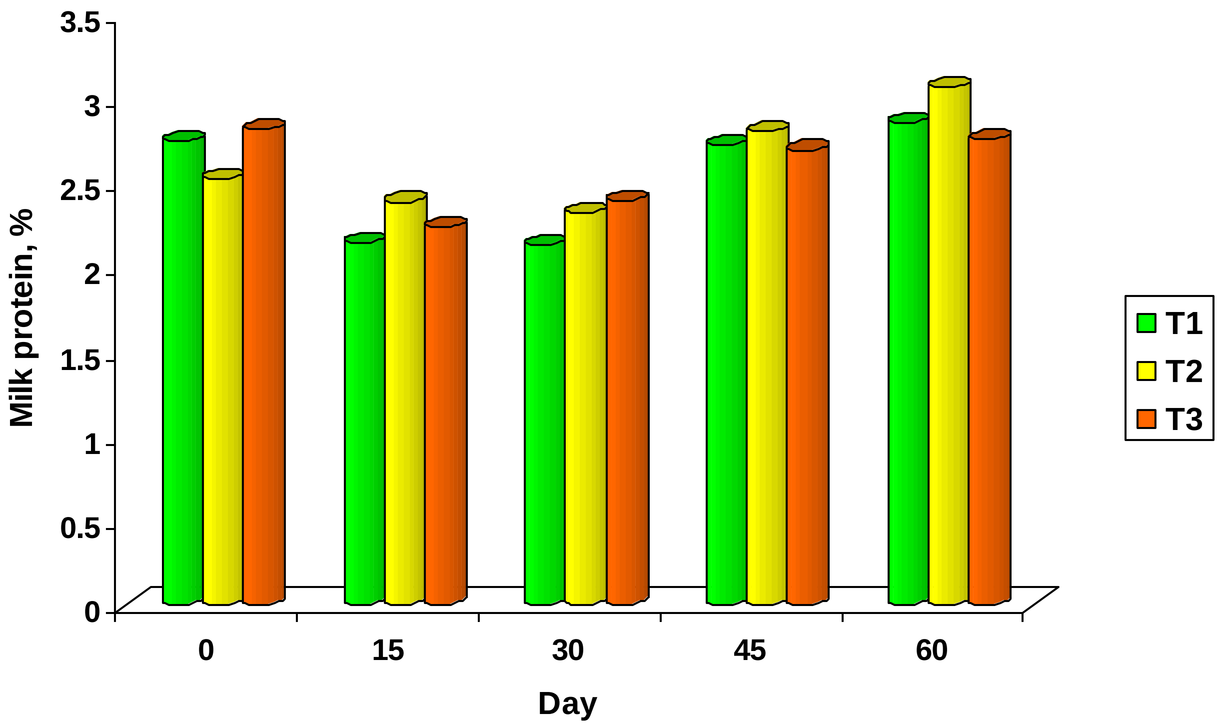


Fig. 7. Average milk protein of experimental cows maintained on three dietary treatments

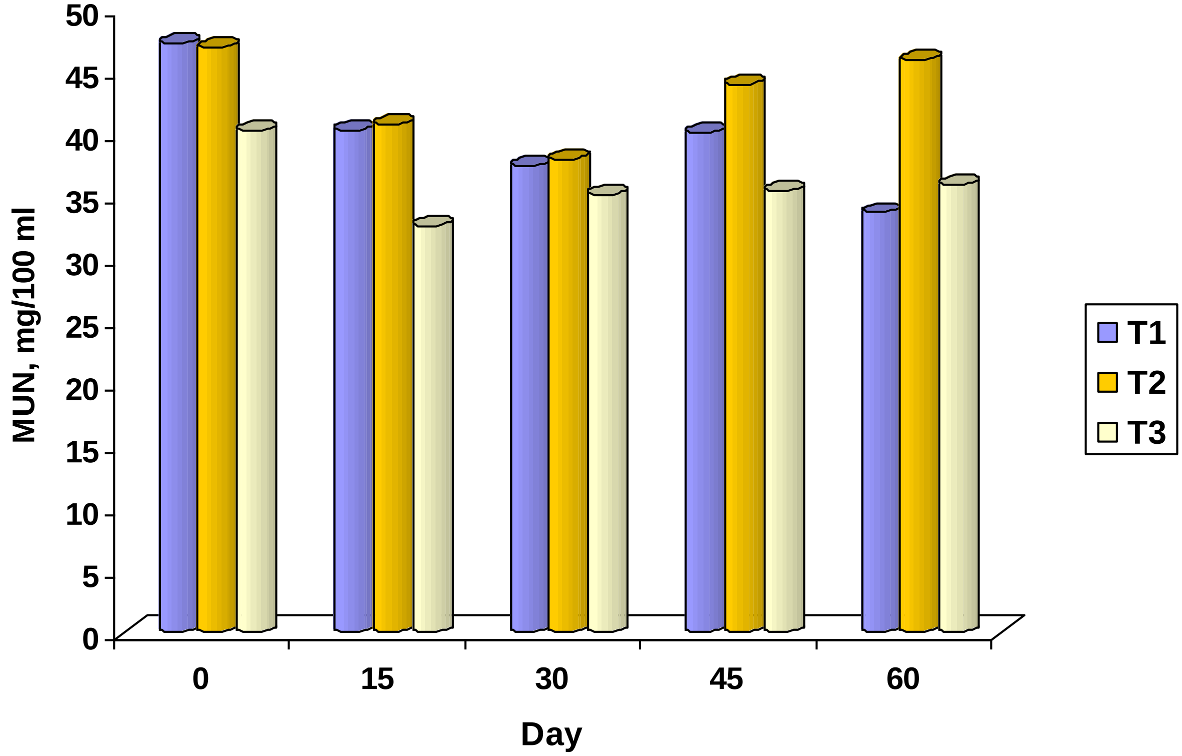


Fig. 8. Average milk urea nitrogen (MUN) of experimental cows maintained on three dietary treatments

Table 10. Yield of four per cent fat corrected milk (FCM), milk fat and milk protein of experimental cows maintained on three dietary treatments for 60 d*, kg

Item	Treatments		
	T1	T2	T3
4 per cent FCM, kg	462.97 ± 20.85	536.89 ± 57.08	533.68 ± 62.49
Milk fat, kg	17.36 ± 1.03	20.01 ± 2.35	19.62 ± 2.30
Milk protein, kg	12.80 ± 0.74	15.50 ± 1.42	15.45 ± 1.87

* Mean of six values ± SE

Table 11. Ruminal pH, total volatile fatty acids and ammonia nitrogen concentration of experimental cows maintained on three dietary treatments*

Treatments	pH	Total volatile fatty acids (meq/l)	Ammonia nitrogen (mg/100ml)
T1	6.96 ± 0.11	101.31 ± 6.67	24.88 ± 2.09
T2	6.75 ± 0.10	99.44 ± 5.04	27.85 ± 3.18
T3	6.87 ± 0.10	94.63 ± 6.03	27.34 ± 1.58

* Mean of six values ± SE

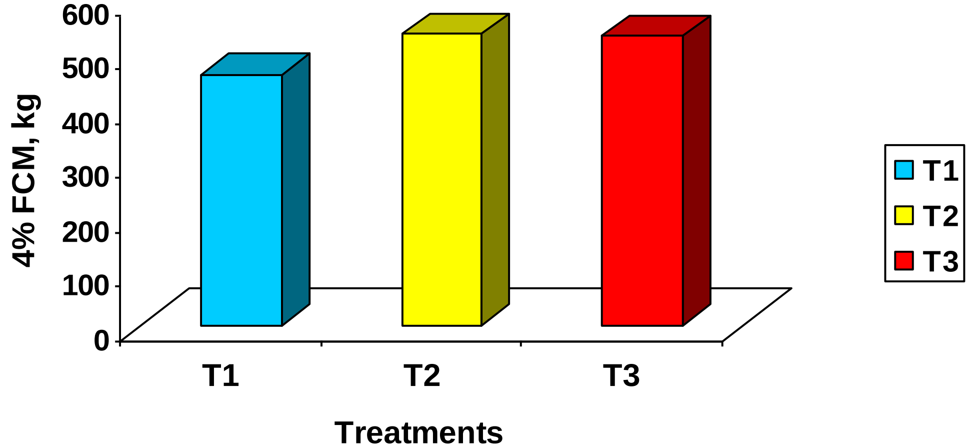


Fig. 9. Four per cent fat corrected milk (FCM) yield of experimental cows maintained on three dietary treatments for 60 d, kg

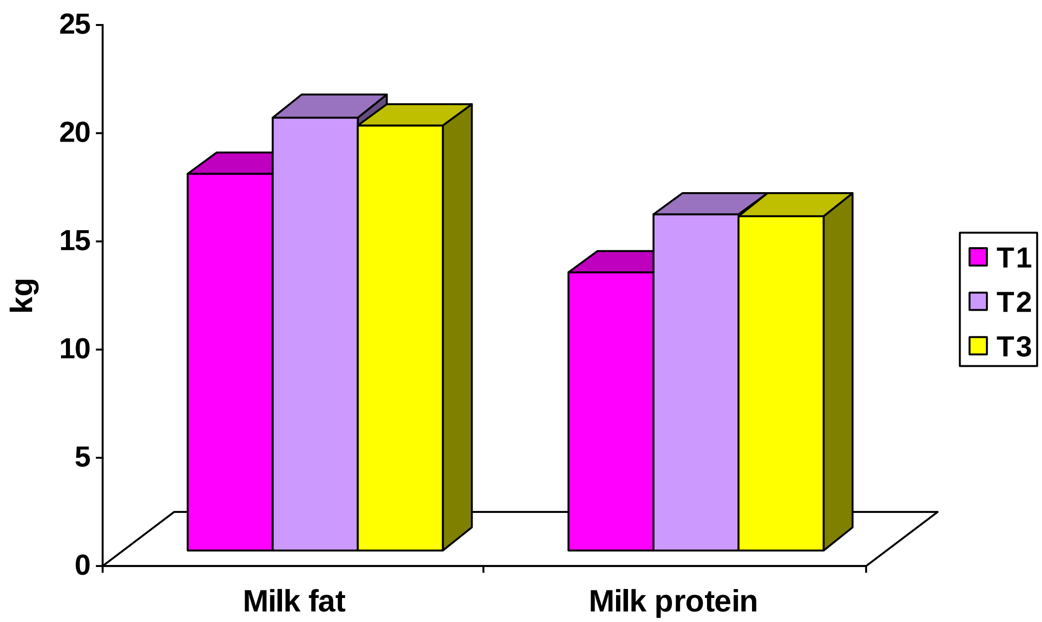


Fig. 10. Yield of milk fat and milk protein of experimental cows maintained on three dietary treatments for 60 d, kg

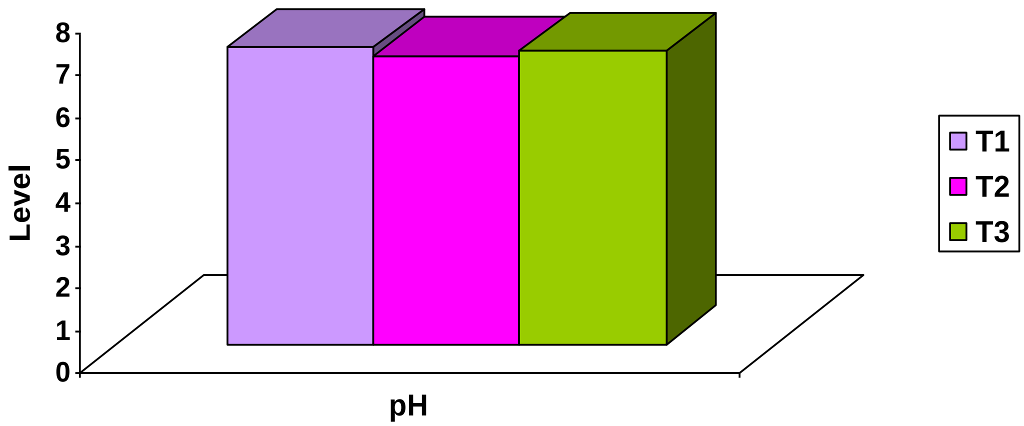


Fig. 11. Average ruminal pH of experimental cows maintained on three dietary treatments

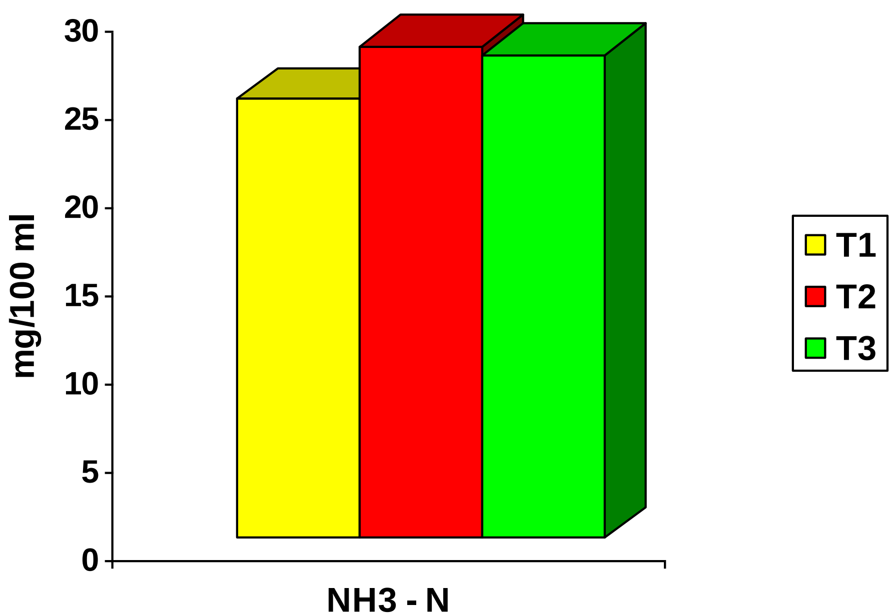


Fig. 12. Average ruminal ammonia nitrogen concentration of experimental cows maintained on three dietary treatments

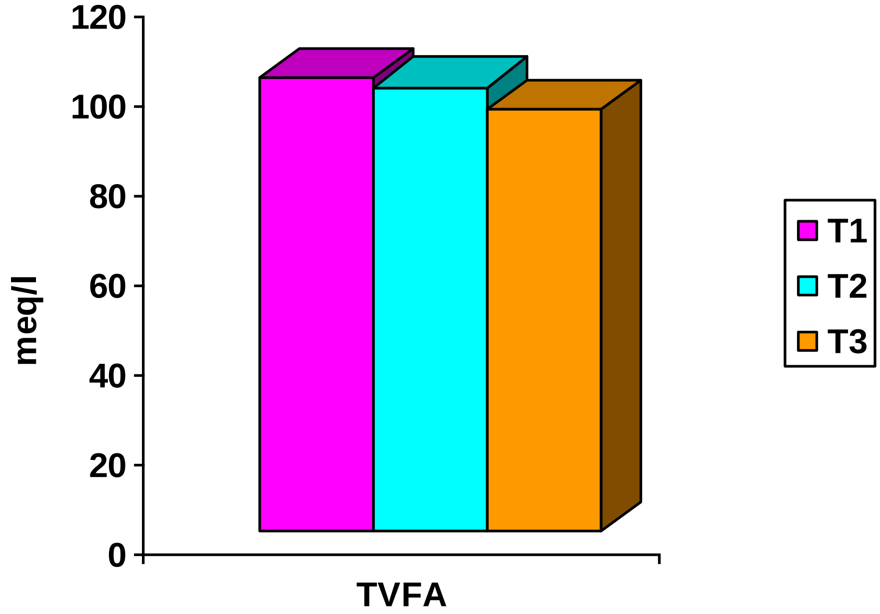


Fig. 13. Average ruminal total volatile fatty acids (TVFA) concentration of experimental cows maintained on three dietary treatments

Table 12. Average haematological parameters of experimental cows maintained on three dietary treatments*

Item	Treatment	Period, d		
		Initial	30	60
Haemoglobin, g %	T1	9.12 ± 0.36	8.79 ± 0.59	8.80 ± 0.37
	T2	8.84 ± 0.30	9.11 ± 0.54	9.22 ± 0.42
	T3	8.92 ± 0.49	9.20 ± 0.38	9.18 ± 0.48
Plasma glucose, mg %	T1	53.75 ± 3.33	52.75 ± 2.07	51.92 ± 2.86
	T2	49.42 ± 2.42	56.58 ± 2.57	54.42 ± 3.39
	T3	52.83 ± 2.34	57.75 ± 2.32	56.42 ± 3.50
Plasma urea nitrogen, mg %	T1	34.25 ± 2.00	27.39 ± 1.67	32.48^{a} ± 2.28
	T2	33.63 ± 1.27	30.24 ± 1.51	41.45^{b} ± 2.00
	T3	32.46 ± 1.32	26.44 ± 1.72	30.10^{a} ± 3.64
Plasma calcium, mg %	T1	9.77 ± 0.67	9.95 ± 0.39	9.87 ± 0.46
	T2	9.64 ± 0.66	10.19 ± 0.44	10.12 ± 0.48
	T3	10.09 ± 0.81	10.10 ± 0.45	10.20 ± 0.45
Plasma phosphorus, mg %	T1	6.17 ± 0.43	6.45 ± 0.36	6.07 ± 0.58
	T2	6.18 ± 0.47	6.22 ± 0.38	6.16 ± 0.25
	T3	5.89 ± 0.21	6.09 ± 0.40	6.63 ± 0.29

* Mean of six values ± SE

a, b – Means with different superscripts within the same column for each parameter differ significantly ($P<0.05$)

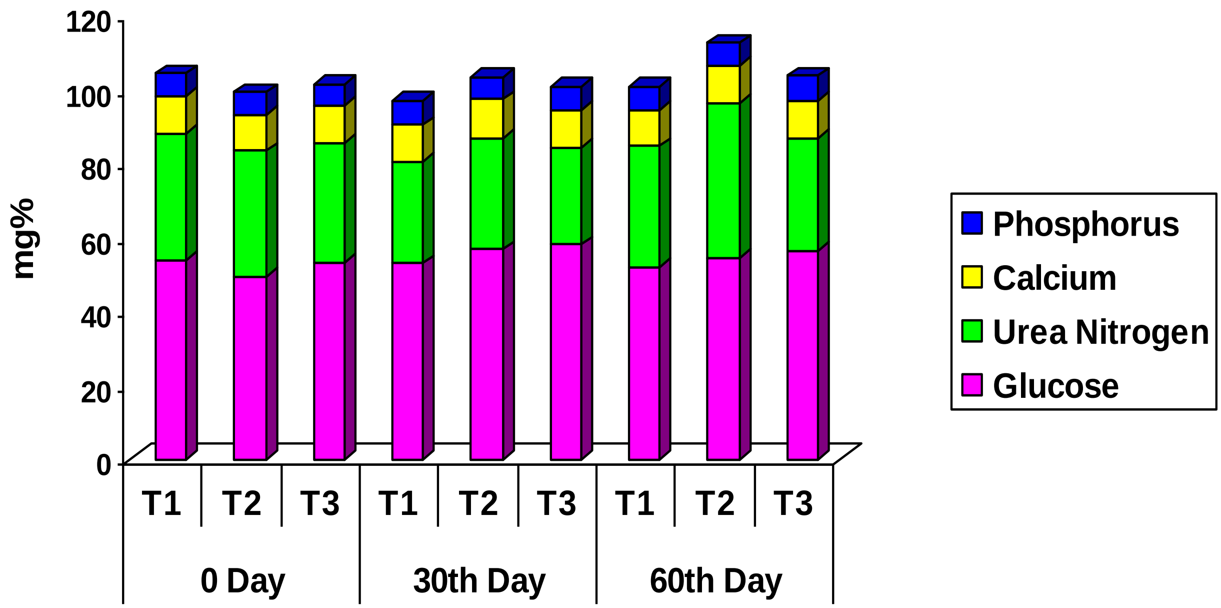


Fig. 14. Average blood parameters of experimental cows maintained on three dietary treatments

Table 13. Chemical composition of dung of experimental cows maintained on three dietary treatments*, %

Item	Treatments		
	T1	T2	T3
Dry matter	19.92 ± 0.35	19.16 ± 0.28	18.41 ± 0.31
Crude protein	9.86 ± 0.61	12.18 ± 0.15	11.50 ± 0.21
Ether extract	2.69 ± 0.30	2.78 ± 0.24	2.46 ± 0.21
Crude fibre	26.57 ± 1.13	24.44 ± 1.36	23.25 ± 1.33
Total ash	11.46 ± 0.94	15.82 ± 0.72	14.48 ± 0.93
Nitrogen free extract	44.70 ± 1.21	41.87 ± 0.58	42.04 ± 1.29
Acid insoluble ash	8.42 ± 0.33	8.65 ± 0.32	8.97 ± 0.46
Neutral detergent fibre	61.01 ± 2.76	58.39 ± 1.86	52.59 ± 2.21
Acid detergent fibre	48.61 ± 0.70	46.56 ± 0.73	46.10 ± 1.09
Hemicellulose	25.17 ± 0.69	25.23 ± 0.79	23.56 ± 1.43
Cellulose	33.86 ± 0.82	34.10 ± 0.56	31.29 ± 0.29
Gross energy** (kcal/kg)	3526.96 ± 34.99	3586.53 ± 10.40	3588.98 ± 11.16
Organic matter	88.54 ± 0.94	84.18 ± 0.72	85.52 ± 0.93

***Mean of six Values** ± SE
**Gross energy determined using Bomb calorimeter (Parr Instrument Company, USA)

Table14. Apparent digestibility of nutrients of three experimental rations*, %

Item	Treatments		
	T1	T2	T3
Dry matter	56.43 ± 2.38	57.90 ± 1.44	54.94 ± 3.08
Crude protein	60.13 ± 2.25	51.71 ± 1.55	52.62 ± 3.27
Crude fibre**	50.27[a] ± 1.03	57.48[b] ± 2.32	56.34[b] ± 1.21
Ether extract	53.28 ± 3.68	51.69 ± 4.28	56.63 ± 2.43
Nitrogen free extract	65.50 ± 1.21	66.78 ± 1.24	65.52 ± 1.59
Neutral detergent fibre**	47.31[a] ± 1.63	54.52[b] ± 1.01	55 .63[b] ± 2.56
Acid detergent fibre	39.10 ± 1.53	43.53 ± 2.05	40.83 ± 3.03
Hemicellulose***	36.85[a] ± 1.89	44.64[b] ± 1.14	46.17[b] ± 1.57
Cellulose	36.95 ± 1.99	38.65 ± 2.50	40.03 ± 3.54
Gross energy	56.16 ± 1.98	57.59 ± 1.46	54.60 ± 3.16
Organic matter	59.12 ± 1.05	60.75 ± 1.07	58.34 ± 2.71

*Mean of six values ± SE

a,b – Means within the same row bearing different superscript vary significantly
** ($P<0.05$); *** ($P<0.01$)

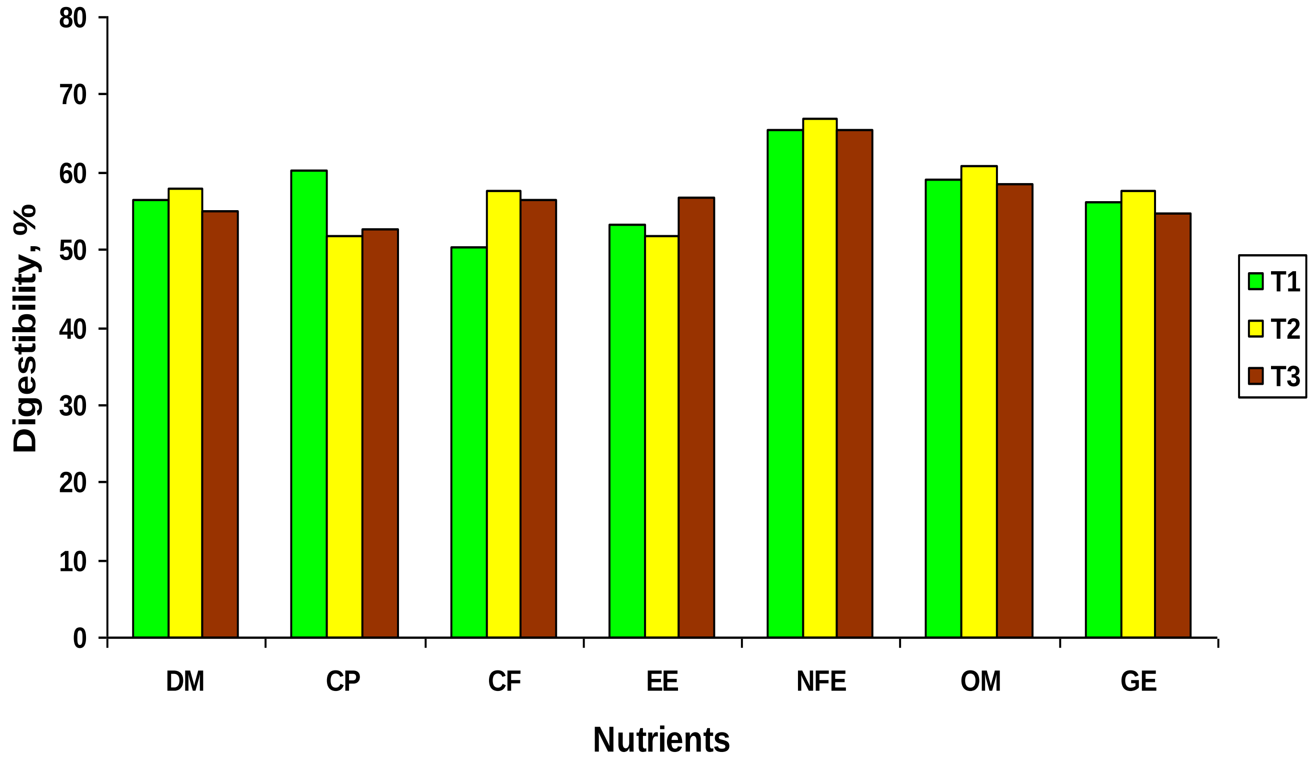


Fig. 15. Apparent digestibility of nutrients of three experimental rations, %

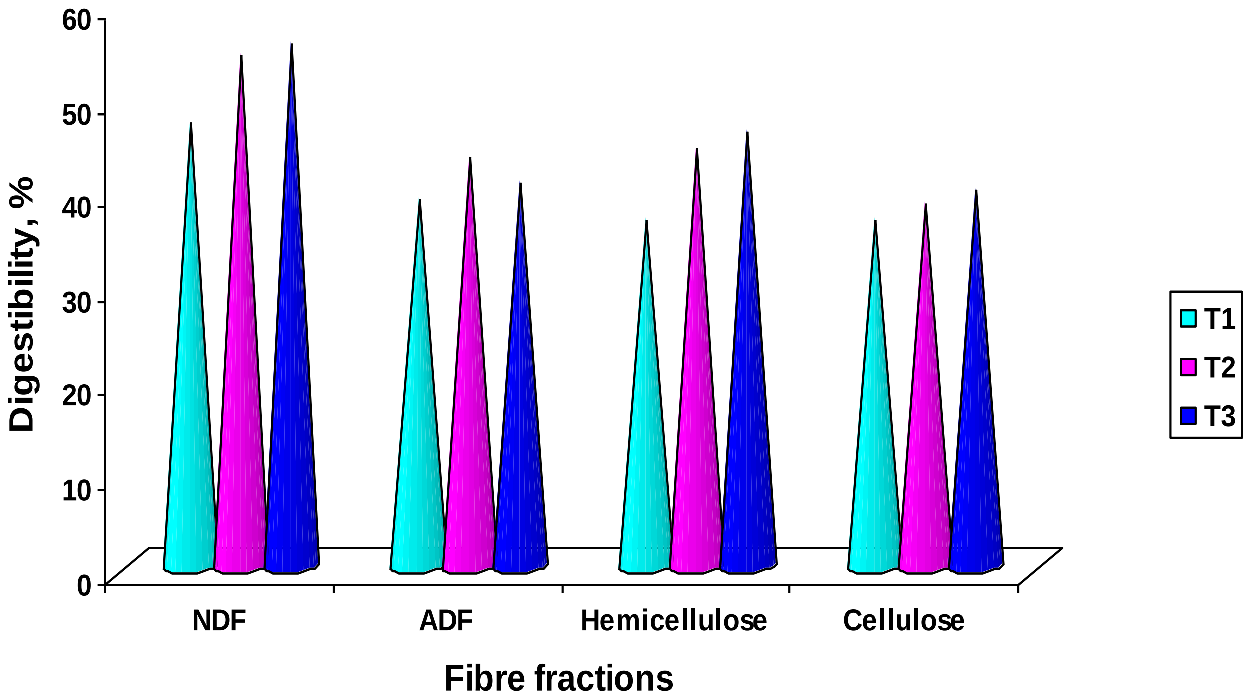


Fig. 16. Apparent digestibility of fibre fractions of three experimental rations, %

Table 15. Total dry matter intake and cost of milk production of experimental cows maintained on three dietary treatments

Item	Treatments		
	T1	T2	T3
Total concentrate mixture consumed, kg	1854.00	1845.00	1939.80
Total paddy straw consumed, kg	3006.00	3060.00	3138.00
Total feed consumed, kg	4860.00	4905.00	5077.80
Dry matter intake (DMI) per 100 kg body weight	3.25	3.11	3.41
Dry matter intake per kg metabolic body weight	14.14	13.81	14.81
Total milk produced in 60 days, kg	2888.50	3651.10	3591.30
Cost of one kg concentrate mixture*, Rs	11.07	10.38	10.38
Cost of one kg paddy straw, Rs	3.16	3.16	3.16
Cost of one kg brewery waste**, Rs	2.00	2.00	2.00
Total cost of feed, Rs.	30023.00	28821.00	30051.00
Cost of feed per kg milk produced, Rs.	10.40	7.89	8.37

* Calculated using the rate contract values fixed for feed ingredients by College of Veterinary and Animal Sciences, Mannuthy, for 2007-2008

** Obtained from UB breweries, Mangalore through Osho farm, Pattikad, Thrissur

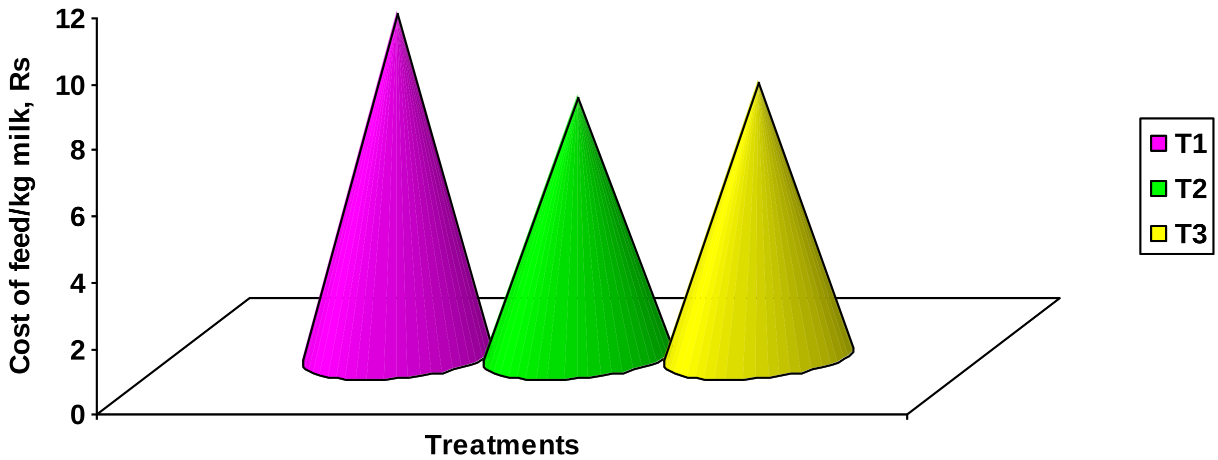


Fig. 17. Cost of milk production of experimental cows maintained on three dietary treatments

Table 16. *In vitro* dry matter disappearance of experimental feeds at different incubation periods (h) in RUSITEC, %

Treatments	DM Disappearance, %						
	0h	2h	6h	12h	24h	48h	72h
Control feed	23.10	26.88	34.39	39.20	46.81	60.37	63.78
Experimental feed with fresh brewery waste (25%)	21.79	25.38	33.04	38.17	45.81	58.89	62.36
Experimental feed with dried brewery waste (25%)	20.05	24.25	31.96	36.13	44.10	56.61	60.70
Brewery waste (fresh)	13.68	16.66	26.57	34.99	41.52	48.94	52.68
Brewery waste (dried)	10.98	16.02	24.51	33.65	40.02	43.07	46.76
Paddy straw	10.33	15.62	22.40	24.35	27.50	30.51	33.24
Brewery waste incorporated paddy straw	11.85	15.73	23.49	28.74	32.00	38.31	42.25

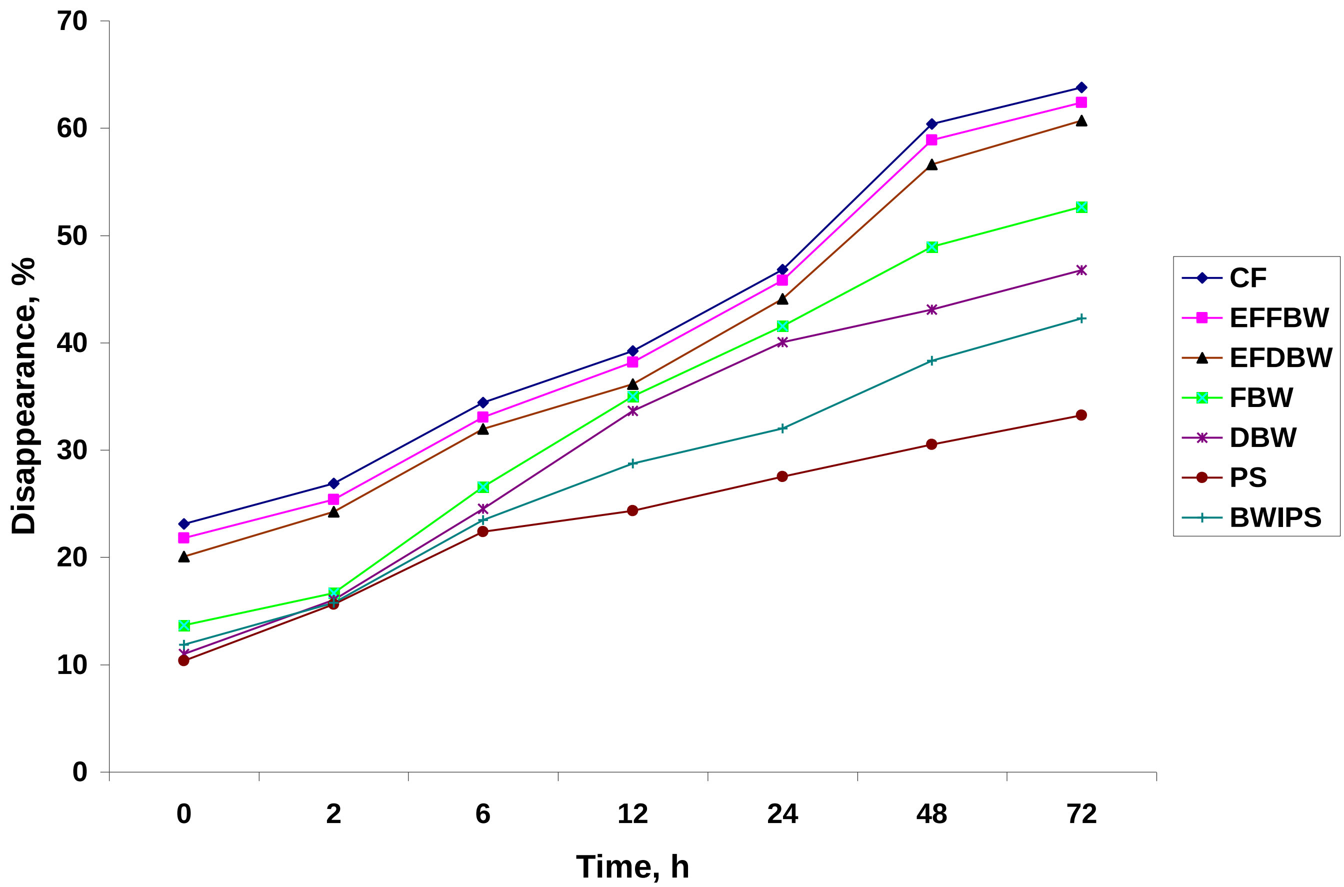


Fig. 18. ***In vitro*** **dry matter disappearance of experimental feeds at different incubation periods (h) in RUSITEC, %**

Table 17. *In vitro* crude protein disappearance of experimental feeds at different incubation periods (h) in RUSITEC, %

Treatments	CP Disappearance, %						
	0h	2h	6h	12h	24h	48h	72h
Control feed	10.87	15.16	24.13	31.18	43.09	53.65	56.06
Experimental feed with fresh brewery waste (25%)	9.31	14.70	22.13	29.84	41.92	52.03	54.49
Experimental feed with dried brewery waste (25%)	9.28	14.16	20.99	27.90	40.83	50.39	52.59
Brewery waste (fresh)	8.80	13.18	20.10	27.10	34.61	41.03	45.46
Brewery waste (dried)	7.71	12.14	18.85	24.11	32.72	37.82	41.01
Paddy straw	6.73	10.44	13.17	17.77	23.46	28.10	30.75
Brewery waste incorporated paddy straw	7.95	11.17	15.90	20.03	28.44	35.21	38.36

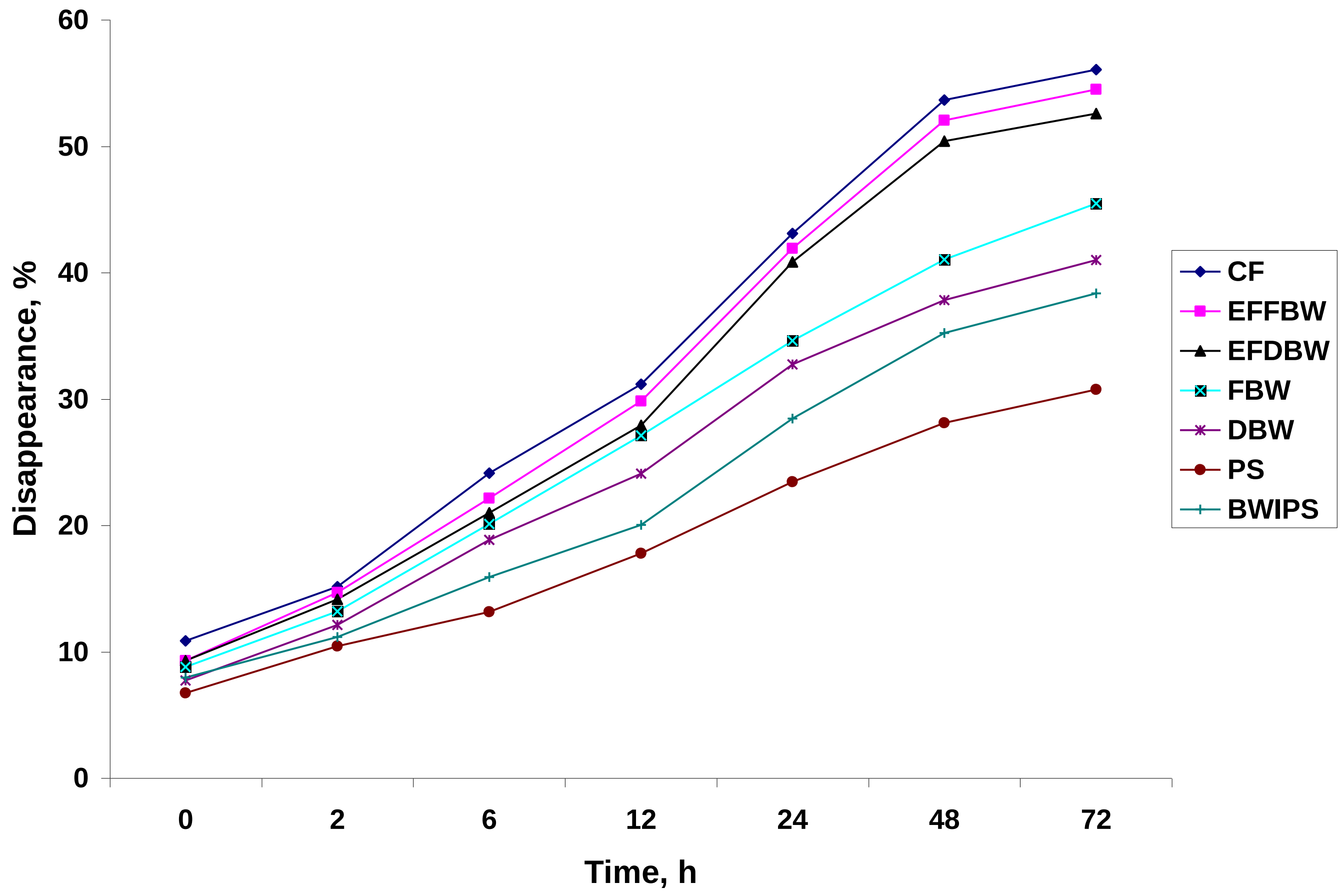


Fig. 19. *In vitro* crude protein disappearance of experimental feeds at different incubation periods (h) in RUSITEC, %

Table 18. *In vitro* crude fibre disappearance of experimental feeds at different incubation periods (h) in RUSITEC, %

Treatments	CF Disappearance, %						
	0h	2h	6h	12h	24h	48h	72h
Control feed	16.30	22.00	30.83	38.86	46.72	59.81	62.54
Experimental feed with fresh brewery waste (25%)	15.61	21.45	30.38	37.38	45.70	58.47	61.67
Experimental feed with dried brewery waste (25%)	15.46	20.35	27.78	35.48	44.22	55.66	60.17
Brewery waste (fresh)	13.18	16.88	26.30	34.67	40.67	48.42	51.26
Brewery waste (dried)	11.70	15.81	24.31	32.96	40.32	42.76	44.87
Paddy straw	10.83	15.03	22.35	24.40	27.41	30.64	33.03
Brewery waste incorporated paddy straw	11.36	15.83	23.18	29.24	32.36	39.27	41.76

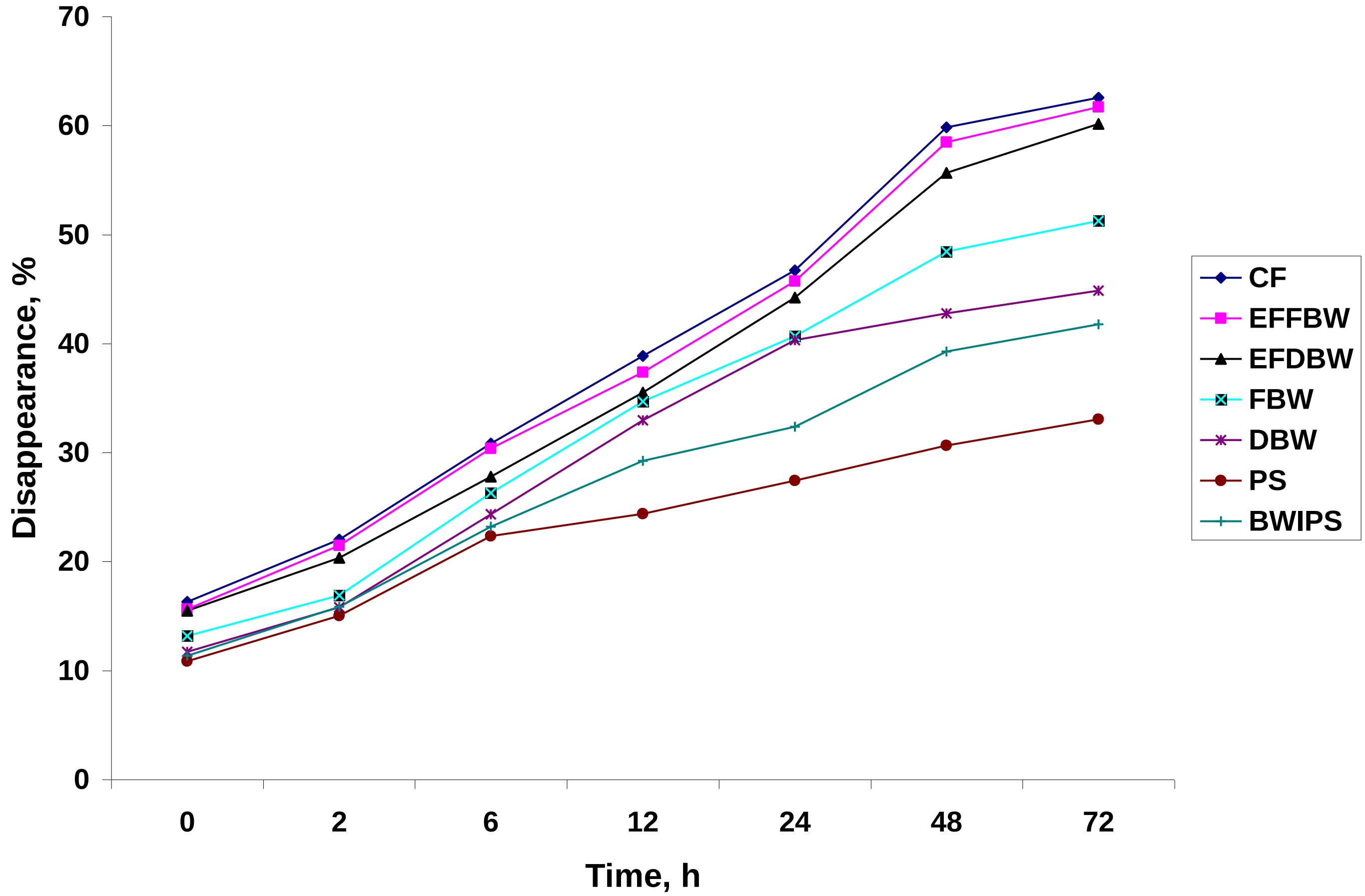


Fig. 20. *In vitro* crude fibre disappearance of experimental feeds at different incubation periods (h) in RUSITEC, %

Table 19. ***In vitro*** **neutral detergent fibre (NDF) disappearance of experimental feeds at different incubation periods (h) in RUSITEC, %**

Treatments	NDF Disappearance, %						
	0h	2h	6h	12h	24h	48h	72h
Control feed	16.94	22.27	33.28	39.37	45.04	50.12	54.89
Experimental feed with fresh brewery waste (25%)	13.79	18.28	25.72	30.81	40.04	51.87	54.33
Experimental feed with dried brewery waste (25%)	11.82	17.19	24.44	31.27	40.84	49.57	52.99
Brewery waste (fresh)	11.76	14.96	20.20	25.33	35.65	41.97	46.45
Brewery waste (dried)	10.56	13.25	18.51	23.46	32.19	39.48	44.16
Paddy straw	9.23	12.57	17.16	21.19	28.51	33.86	36.66
Brewery waste incorporated paddy straw	10.71	14.16	20.32	26.67	34.42	38.20	41.09

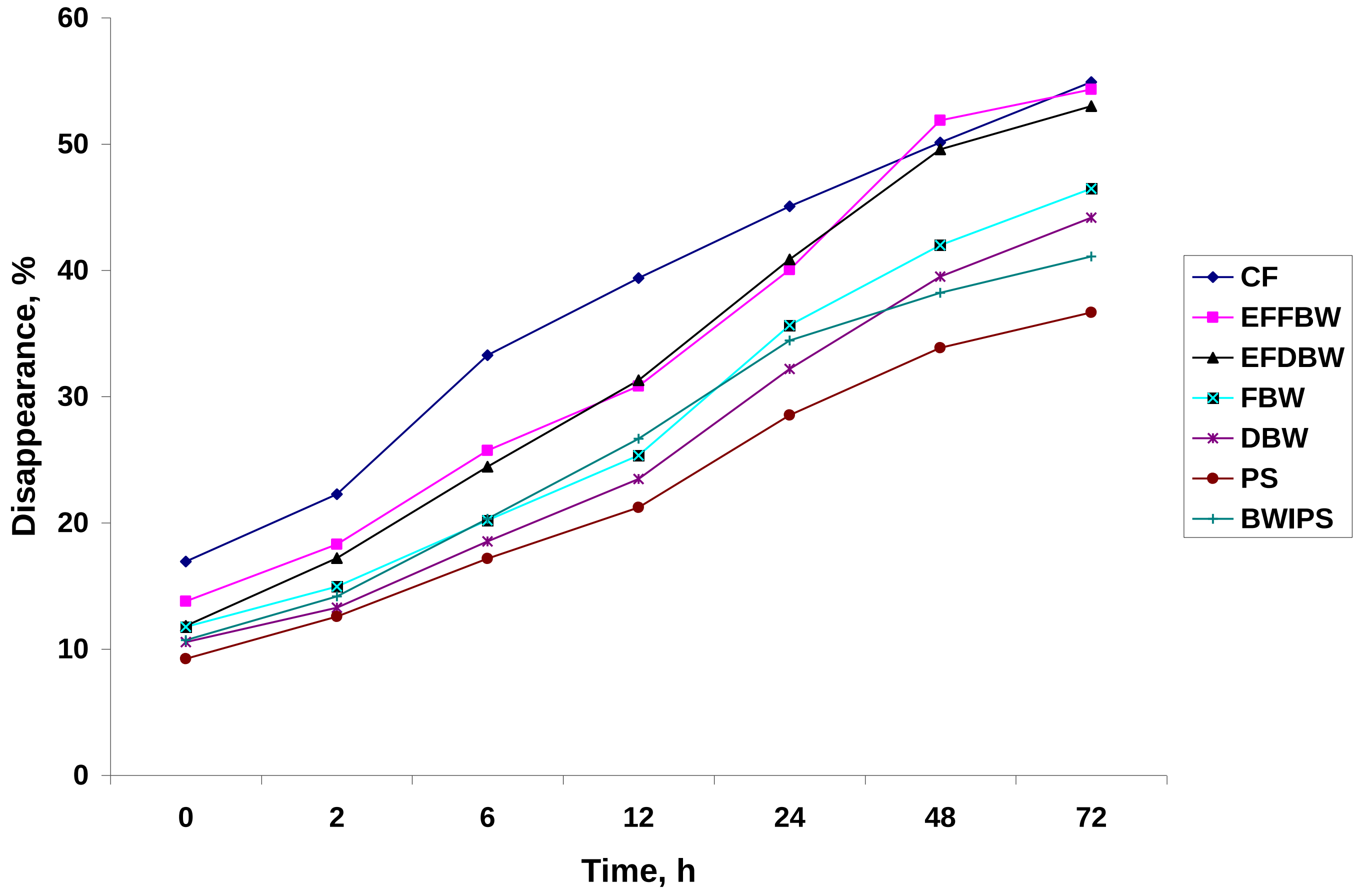


Fig. 21. ***In vitro*** **neutral detergent fibre (NDF) disappearance of experimental feeds at different incubation periods (h) in RUSITEC, %**

Table 20. *In vitro* acid detergent fibre (ADF) disappearance of experimental feeds at different incubation periods (h) in RUSITEC, %

Treatments	ADF Disappearance, %						
	0h	2h	6h	12h	24h	48h	72h
Control feed	12.56	20.34	28.97	37.18	41.76	48.29	53.02
Experimental feed with fresh brewery waste (25%)	10.81	15.46	22.39	29.06	35.55	48.99	51.87
Experimental feed with dried brewery waste (25%)	9.70	14.81	20.71	27.82	32.89	48.31	51.09
Brewery waste (fresh)	9.43	13.95	20.54	27.85	34.43	43.72	45.52
Brewery waste (dried)	8.57	12.67	18.45	24.95	31.42	41.54	43.76
Paddy straw	8.27	11.45	16.34	20.56	26.75	32.92	35.28
Brewery waste incorporated paddy straw	9.16	13.24	19.00	25.40	33.28	37.97	39.69

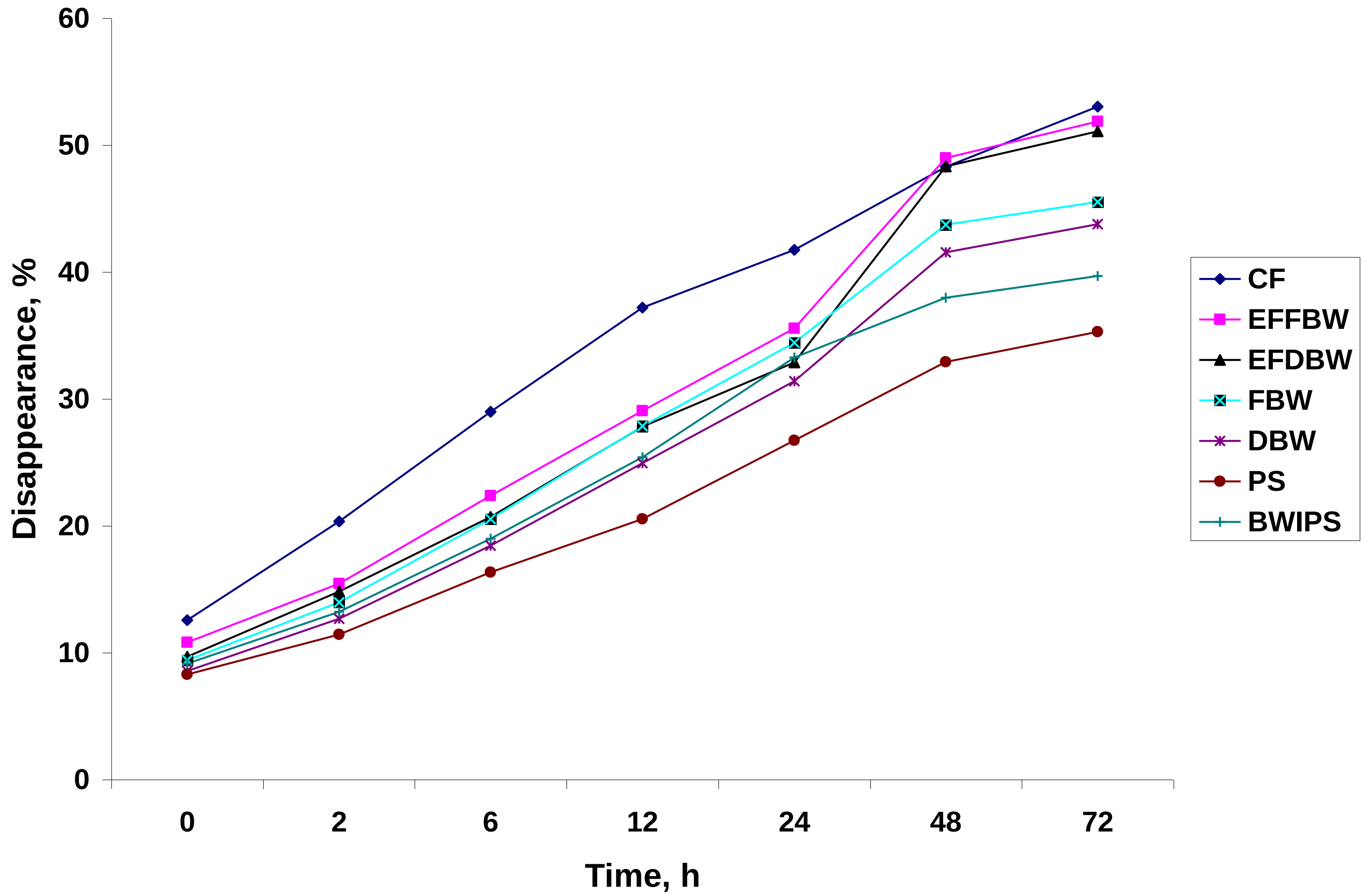


Fig. 22. ***In vitro*** **acid detergent fibre (ADF) disappearance of experimental feeds at different incubation periods (h) in RUSITEC, %**

Table 21. *In vitro* hemicellulose disappearance of experimental feeds at different incubation periods (h) in RUSITEC, %

Treatments	Hemicellulose Disappearance, %						
	0h	2h	6h	12h	24h	48h	72h
Control feed	13.99	20.54	30.42	37.55	42.62	51.19	54.84
Experimental feed with fresh brewery waste (25%)	11.86	15.97	22.48	29.89	36.99	50.48	53.89
Experimental feed with dried brewery waste (25%)	10.17	14.88	21.56	27.89	36.76	49.55	52.10
Brewery waste (fresh)	8.95	14.27	20.38	27.64	33.73	43.34	46.62
Brewery waste (dried)	8.01	12.83	19.15	24.28	30.87	40.40	44.31
Paddy straw	8.00	11.90	16.89	21.17	28.44	33.58	37.11
Brewery waste incorporated paddy straw	9.21	13.90	18.74	25.53	33.92	35.41	39.35

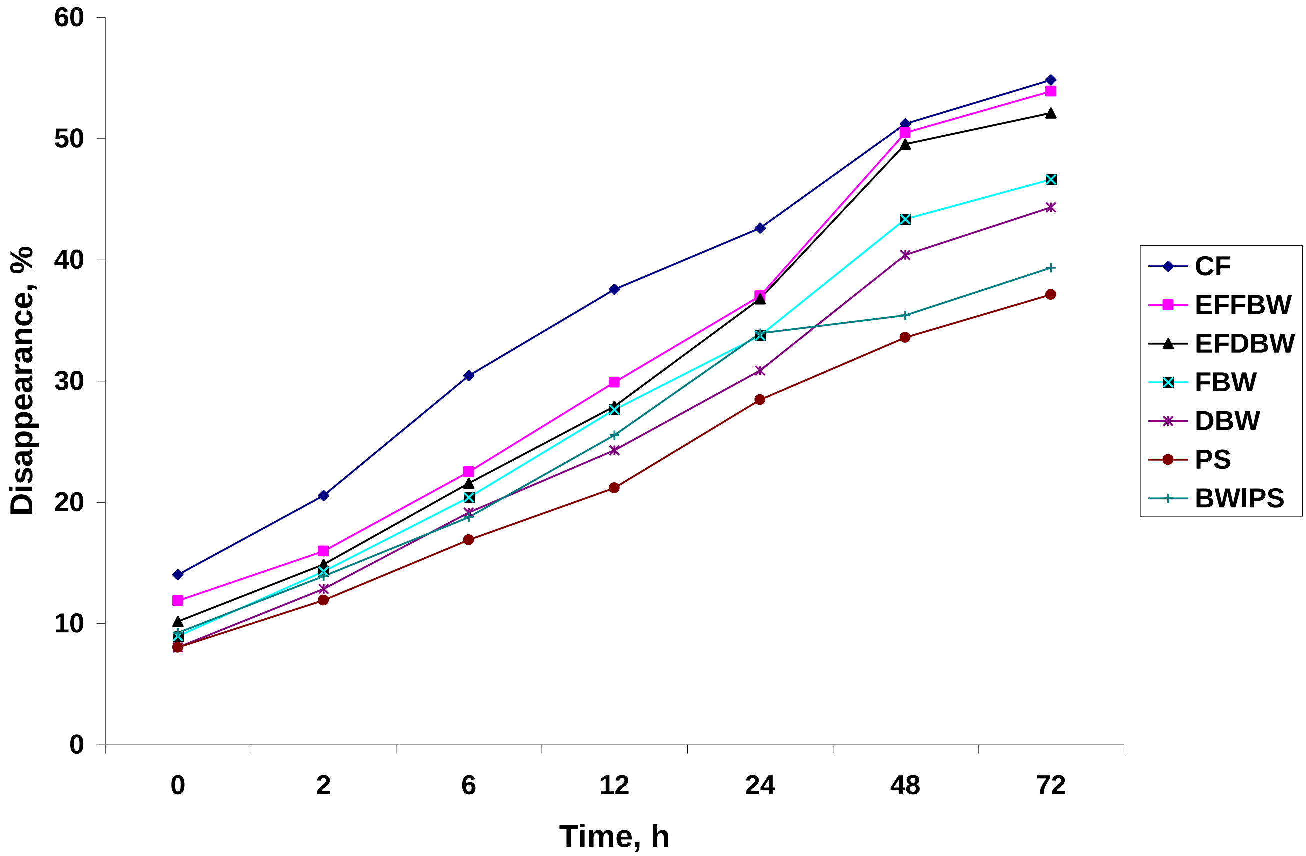


Fig. 23. ***In vitro*** **hemicellulose disappearance of experimental feeds at different incubation periods (h) in RUSITEC, %**

Table 22. *In vitro* cellulose disappearance of experimental feeds at different incubation periods (h) in RUSITEC, %

Treatments	Cellulose Disappearance, %						
	0h	2h	6h	12h	24h	48h	72h
Control feed	12.05	18.73	29.50	37.62	43.70	50.16	52.50
Experimental feed with fresh brewery waste (25%)	9.82	14.42	20.95	27.58	34.53	47.81	51.61
Experimental feed with dried brewery waste (25%)	9.22	13.96	19.82	25.34	31.80	47.13	50.99
Brewery waste (fresh)	8.53	13.63	20.09	27.21	32.85	42.93	46.24
Brewery waste (dried)	8.04	11.93	17.28	25.55	30.88	39.24	42.19
Paddy straw	5.52	8.43	12.97	17.97	22.82	28.63	30.36
Brewery waste incorporated paddy straw	7.41	12.10	16.93	23.45	31.01	35.69	37.84

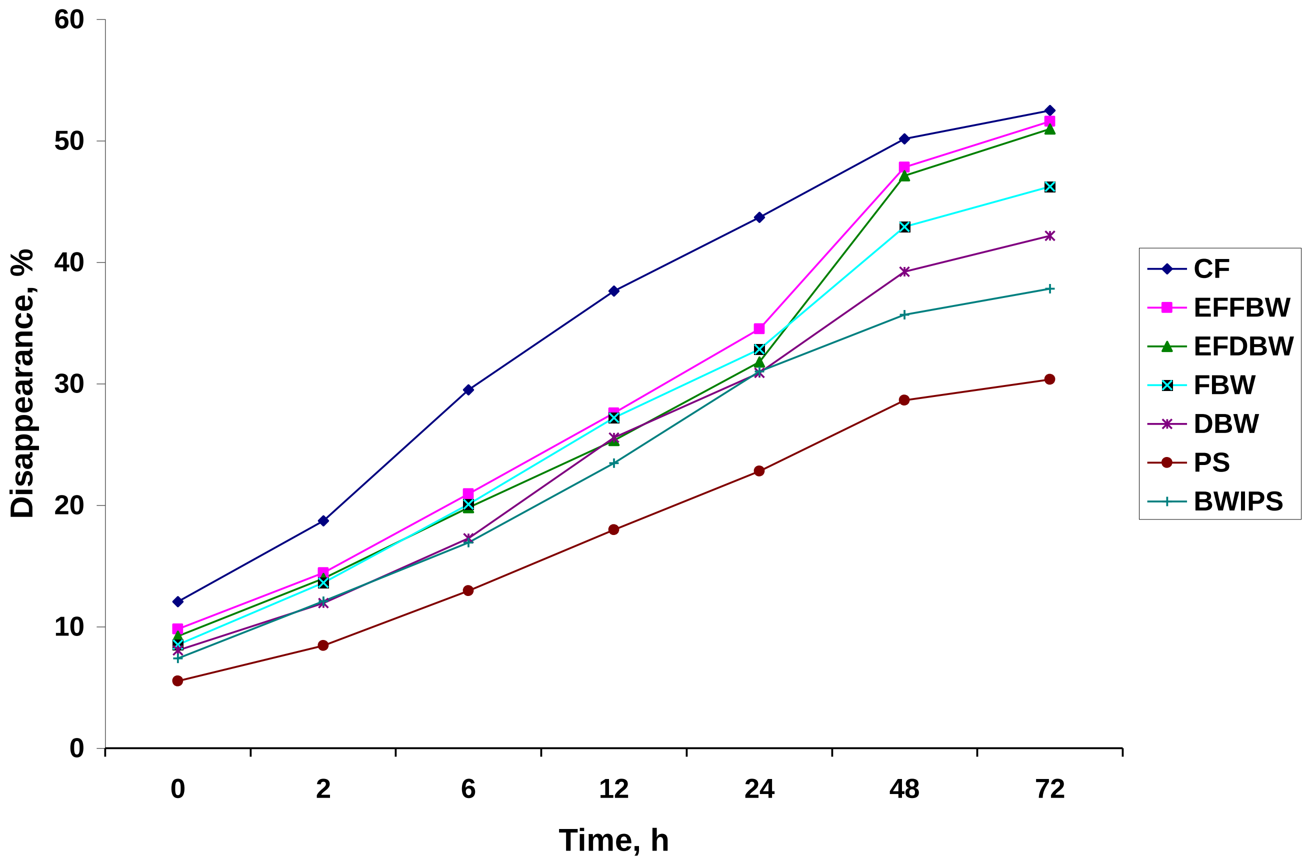


Fig. 24. *In vitro* cellulose disappearance of experimental feeds at different incubation periods (h) in RUSITEC, %

Table 23. *In vitro* effective dry matter degradability of experimental feeds in RUSITEC, %

Treatments	Soluble 'a', %	Degradable 'b', %	Undegradable, %	Rate of degradation 'c', % / h	Effective degradability, %	Escape, %
Control feed	23.10	40.68	36.22	0.51	61.74	38.26
Experimental feed with fresh brewery waste (25%)	21.79	40.57	37.64	0.51	60.32	39.68
Experimental feed with dried brewery waste (25%)	20.05	40.65	39.30	0.50	58.62	41.38
Brewery waste (fresh)	13.68	39.00	47.32	0.45	50.48	49.52
Brewery waste (dried)	10.98	35.78	53.24	0.37	44.34	55.66
Paddy straw	10.33	22.91	66.76	0.21	30.59	69.41
Brewery waste incorporated paddy straw	11.85	30.40	57.75	0.33	39.93	60.07

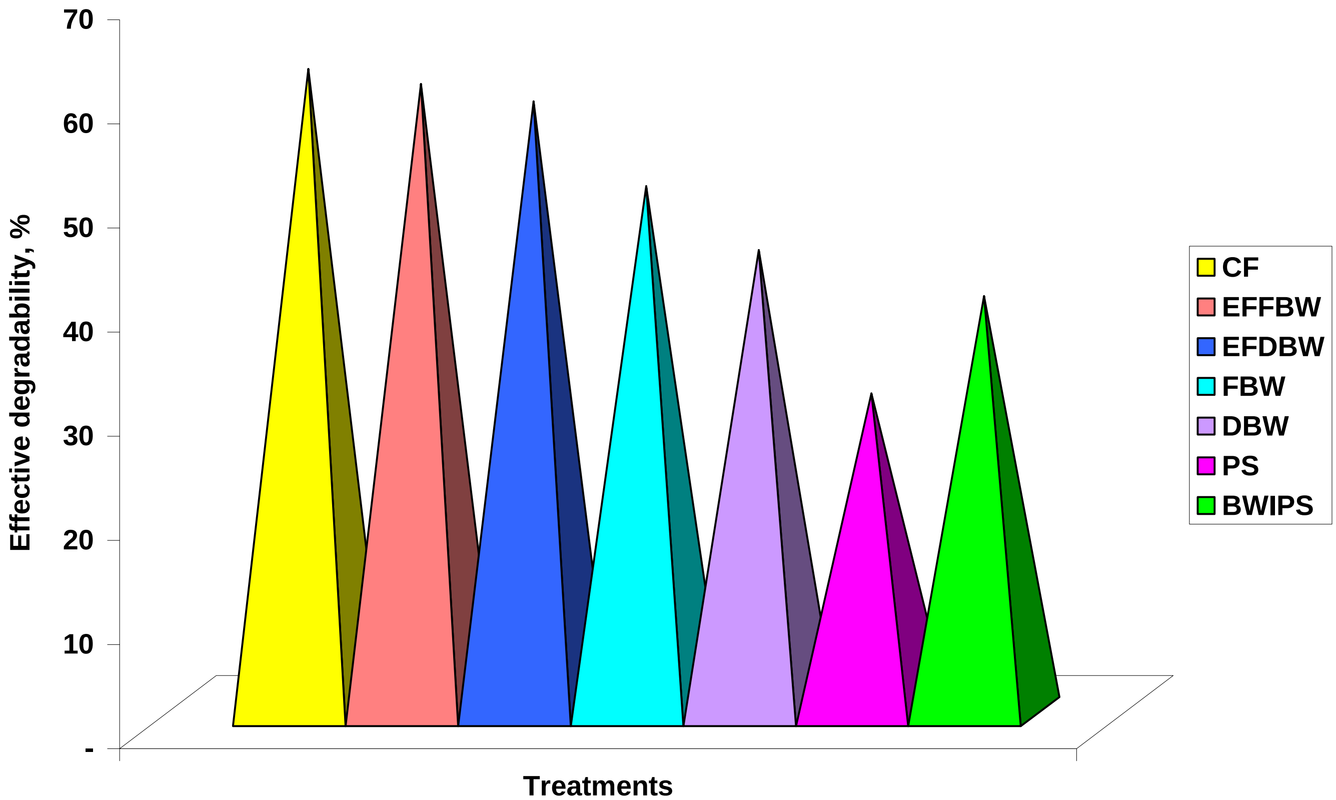


Fig. 25. ***In vitro*** **effective dry matter degradability of experimental feeds in RUSITEC, %**

Table 24. ***In vitro*** **effective crude protein degradability of experimental feeds in RUSITEC, %**

Treatments	Soluble 'a', %	Degradable 'b', %	Undegradable, %	Rate of degradation 'c', % / h	Effective degradability, %	Escape, %
Control feed	10.87	45.19	43.94	0.56	53.97	46.03
Experimental feed with fresh brewery waste (25%)	9.31	45.18	45.51	0.55	52.37	47.63
Experimental feed with dried brewery waste (25%)	9.28	43.31	47.41	0.54	50.51	49.49
Brewery waste (fresh)	8.80	36.66	54.54	0.42	43.25	56.75
Brewery waste (dried)	7.70	33.30	58.99	0.38	38.79	61.21
Paddy straw	6.73	24.02	69.25	0.28	28.64	71.36
Brewery waste incorporated paddy straw	7.95	30.41	61.64	0.38	36.32	63.68

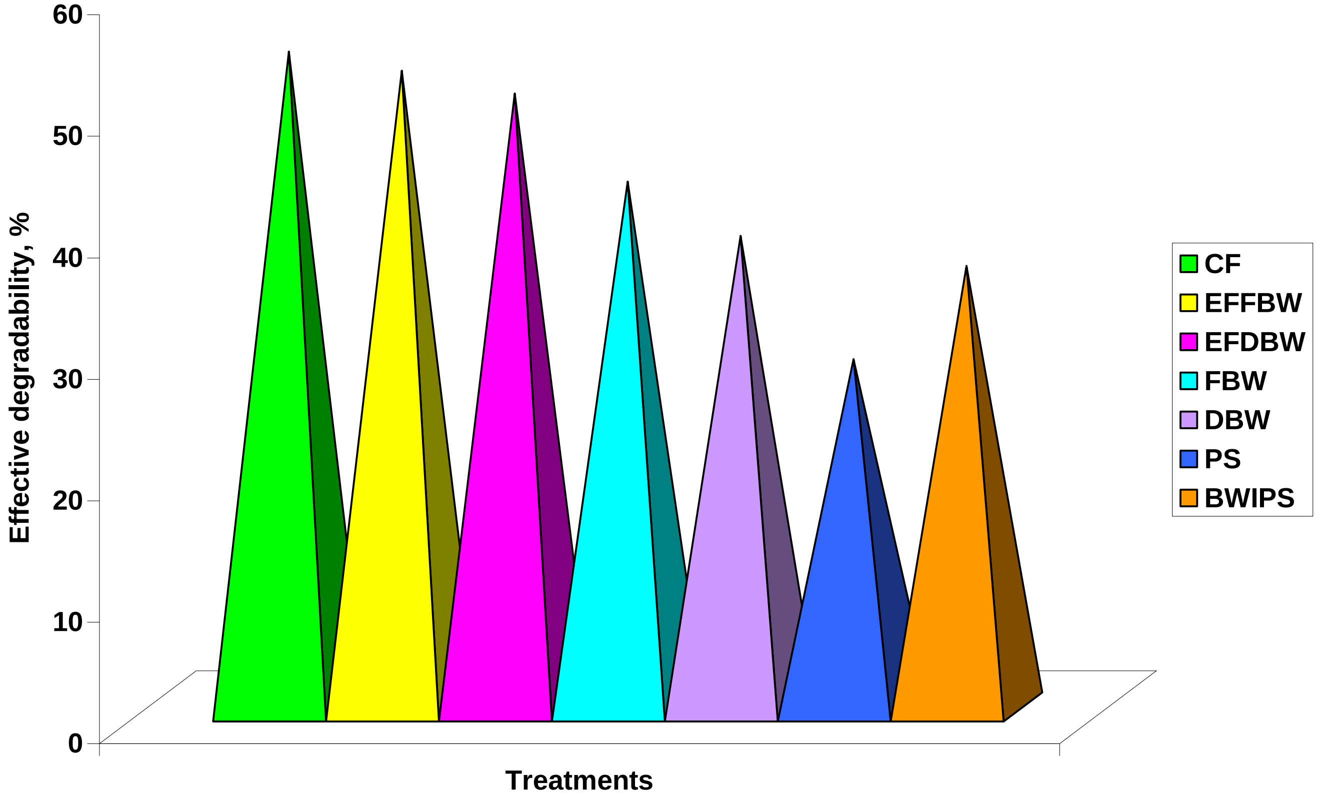


Fig. 26. *In vitro* effective crude protein degradability of experimental feeds in RUSITEC, %

Table 25. *In vitro* total gas production of experimental feeds at different incubation periods (h) in RUSITEC, ml

Treatments	Total Gas Production, ml					
	2h	6h	12h	24h	48h	72h
Control feed	330	835	1095	1800	1975	2486
Experimental feed with fresh brewery waste (25%)	300	680	930	1650	2786	3925
Experimental feed with dried brewery waste (25%)	350	715	860	1545	2525	3860
Brewery waste (fresh)	395	585	645	1580	2035	2415
Brewery waste (dried)	133	230	565	975	2310	3585
Paddy straw	155	220	455	1045	1395	2435
Brewery waste incorporated paddy straw	156	290	578	1293	2200	3115

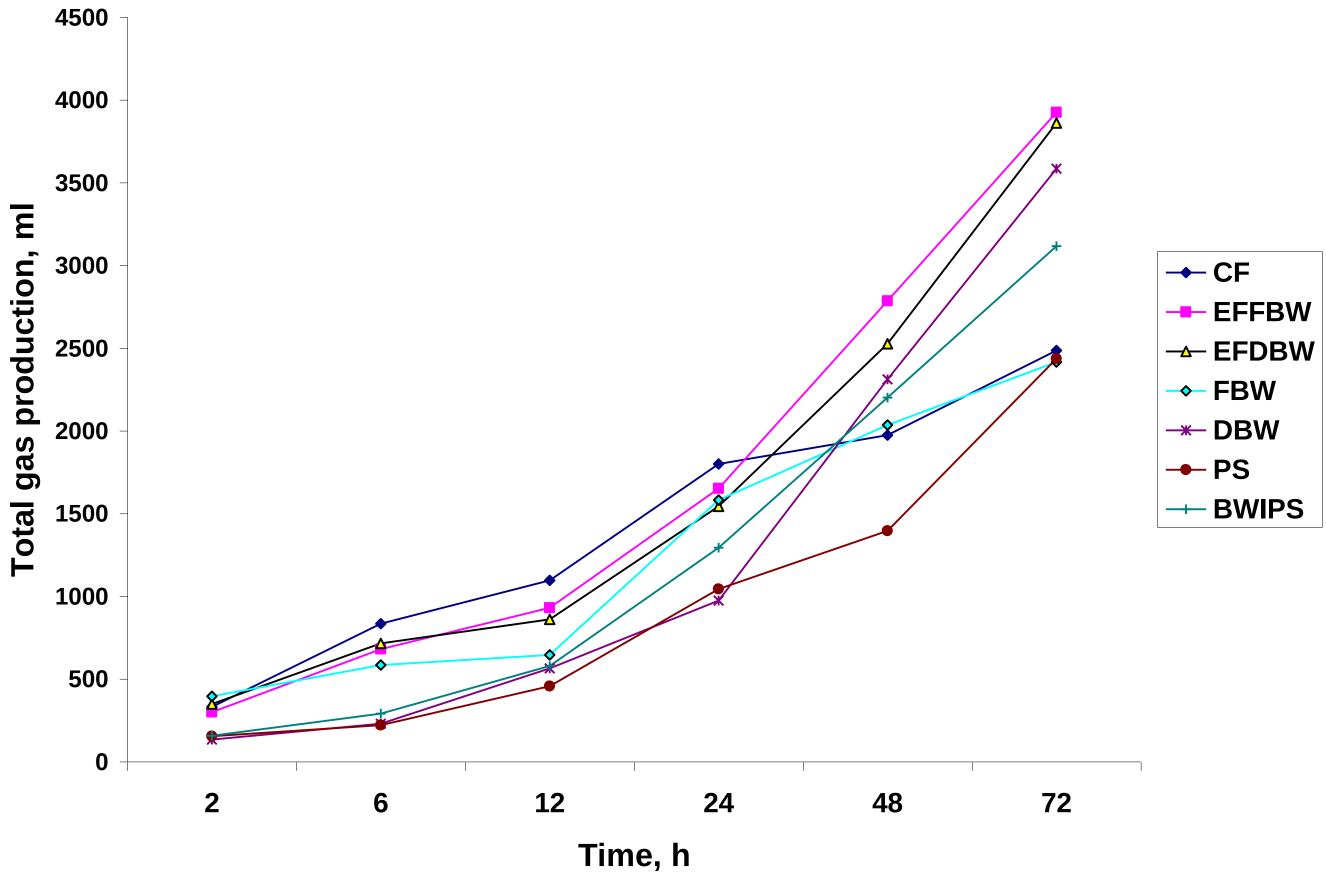


Fig. 27. ***In vitro*** **total gas production of experimental feeds at different incubation periods (h)**

in RUSITEC, ml

Table 26. ***In vitro*** **carbon dioxide production of experimental feeds at different incubation periods (h) in RUSITEC, %**

Treatments	CO_2, %					
	2h	6h	12h	24h	48h	72h
Control feed	57.50	50.00	60.00	55.00	60.00	57.50
Experimental feed with fresh brewery waste (25%)	57.50	52.50	55.00	55.00	55.00	60.00
Experimental feed with dried brewery waste (25%)	55.00	60.00	57.50	55.00	55.00	57.50
Brewery waste (fresh)	55.00	50.00	52.50	52.50	55.00	52.50
Brewery waste (dried)	55.00	50.00	52.50	55.00	57.50	57.50
Paddy straw	47.50	55.00	60.00	55.00	57.50	57.50
Brewery waste incorporated paddy straw	48.75	52.50	53.75	50.00	53.75	50.00

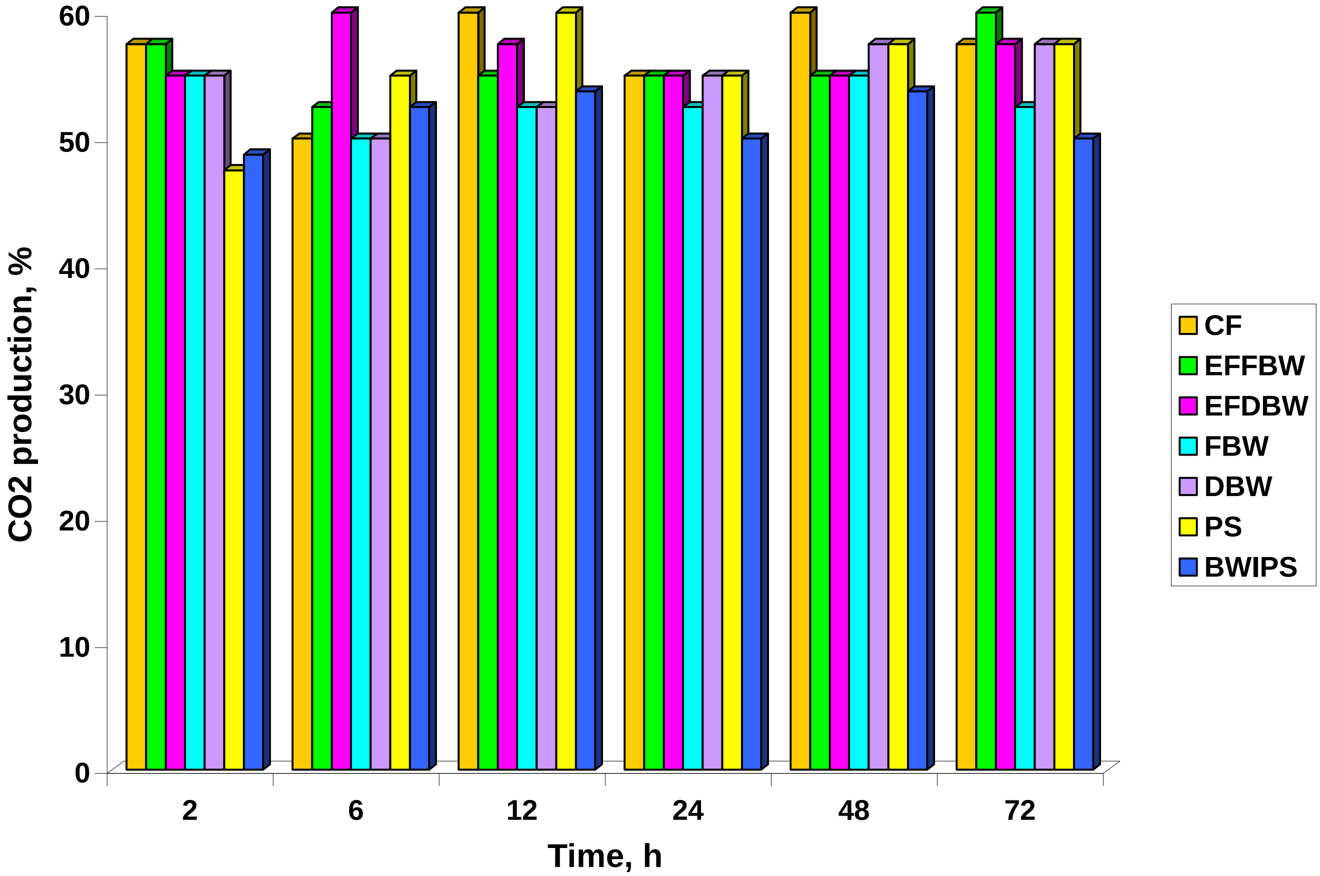


Fig. 28. ***In vitro*** **carbon dioxide production of experimental feeds at different incubation periods (h) in RUSITEC, %**

Table 27. *In vitro* ruminal pH of experimental feeds at different incubation periods (h) in RUSITEC

Treatments	pH					
	2h	6h	12h	24h	48h	72h
Control feed	7.04	7.20	7.28	7.16	7.24	7.20
Experimental feed with fresh brewery waste (25%)	7.21	7.19	7.29	7.04	7.17	7.00
Experimental feed with dried brewery waste (25%)	7.23	7.45	7.37	7.06	7.16	7.19
Brewery waste (fresh)	7.31	7.25	7.28	6.96	7.27	7.08
Brewery waste (dried)	7.22	7.41	6.76	7.26	7.42	6.98
Paddy straw	7.00	7.30	7.04	7.02	7.11	7.02
Brewery waste incorporated paddy straw	7.24	7.23	6.89	7.00	7.21	6.94

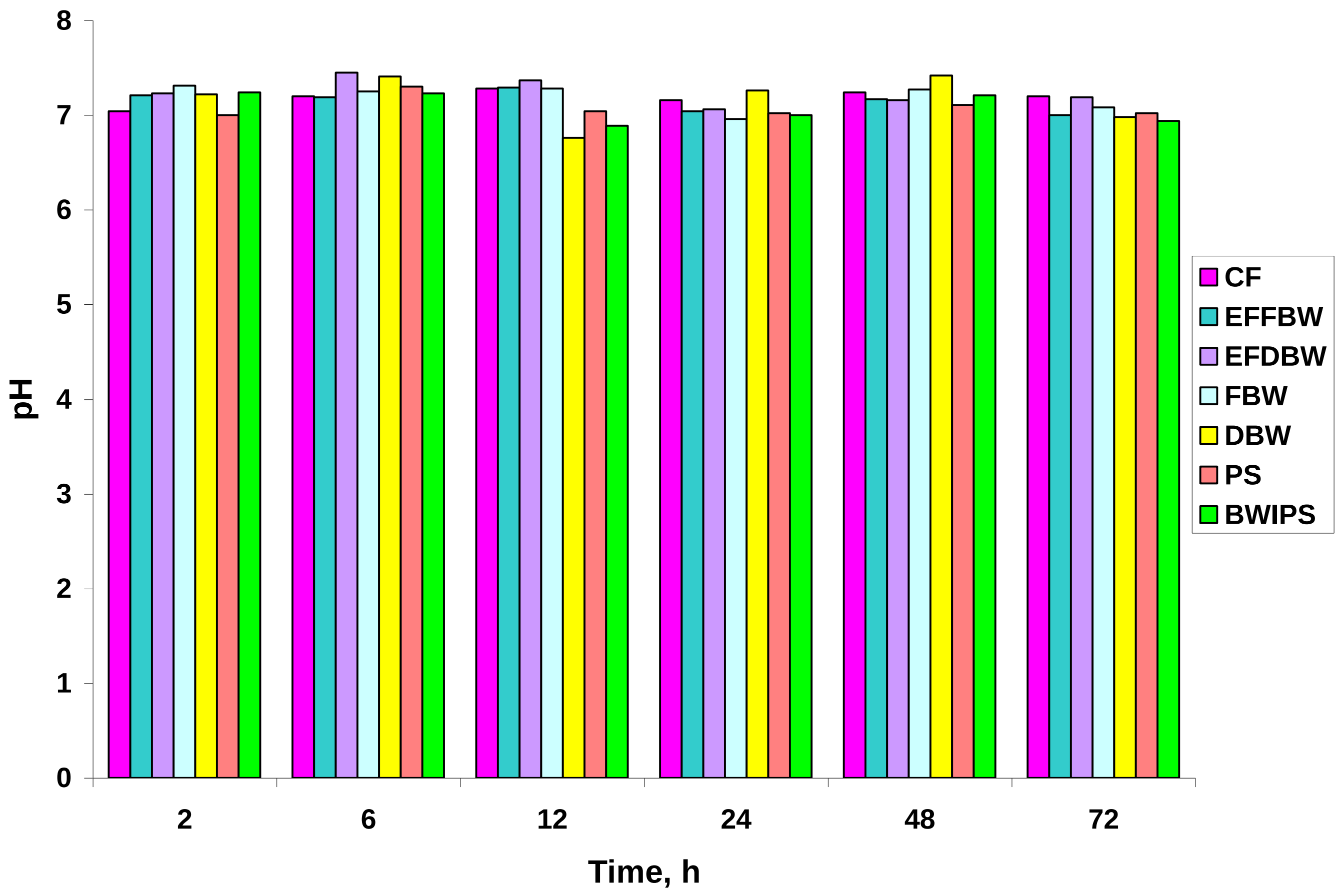


Fig. 29. ***In vitro*** **ruminal pH of experimental feeds at different incubation periods (h) in RUSITEC**

Table 28. *In vitro* ruminal ammonia nitrogen (NH_3-N) production of experimental feeds at different incubation periods (h) in RUSITEC, mg/100 ml

Treatments	NH_3- N, mg/100 ml					
	2h	6h	12h	24h	48h	72h
Control feed	17.12	21.83	18.99	25.64	13.11	20.28
Experimental feed with fresh brewery waste (25%)	19.79	17.60	18.70	16.14	16.76	17.20
Experimental feed with dried brewery waste (25%)	12.64	17.17	15.32	11.38	15.11	9.48
Brewery waste (fresh)	34.92	29.75	25.47	33.15	28.31	32.64
Brewery waste (dried)	11.48	15.69	10.36	9.63	8.57	11.27
Paddy straw	0.66	1.11	0.39	0.70	0.66	0.98
Brewery waste incorporated paddy straw	4.33	13.17	4.42	3.37	1.66	2.22

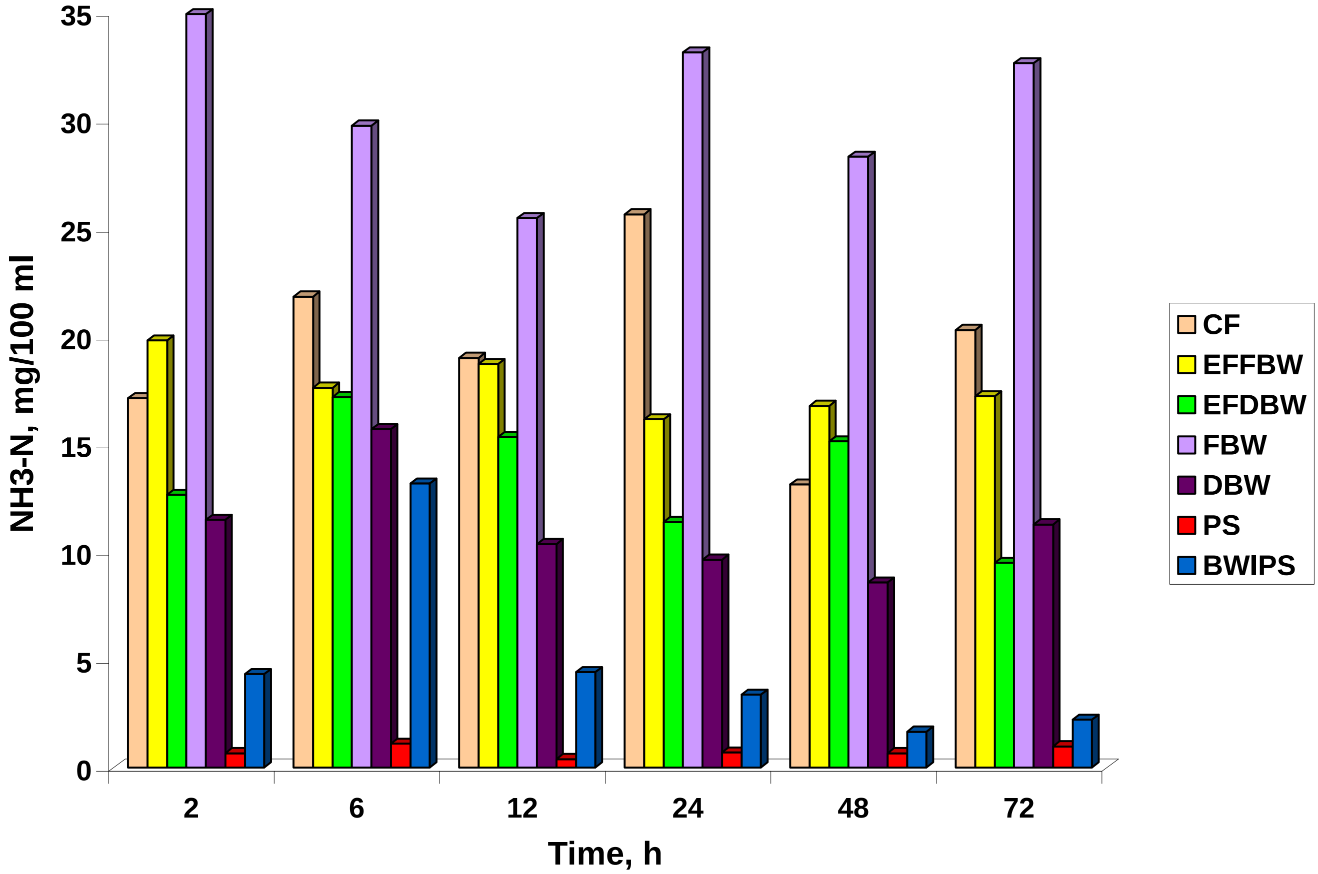


Fig. 30. ***In vitro*** **ruminal ammonia nitrogen (NH_3-N) production of experimental feeds at different incubation periods (h) in RUSITEC, mg/100ml**

Table 29. *In vitro* ruminal total volatile fatty acid (TVFA) production of experimental feeds at different incubation periods (h) in RUSITEC, mmol/l

Treatments	TVFA, mmol/l					
	2h	6h	12h	24h	48h	72h
Control feed	74.25	53.00	63.25	45.25	59.25	64.00
Experimental feed with fresh brewery waste (25%)	40.75	48.50	66.50	49.00	54.00	44.75
Experimental feed with dried brewery waste (25%)	39.00	43.50	66.00	67.25	60.00	59.50
Brewery waste (fresh)	27.00	30.75	33.50	51.25	43.25	41.75
Brewery waste (dried)	29.75	30.75	29.75	34.75	34.00	29.00
Paddy straw	27.50	29.75	29.25	30.50	31.25	29.75
Brewery waste incorporated paddy straw	27.00	35.38	29.00	29.88	31.88	27.13

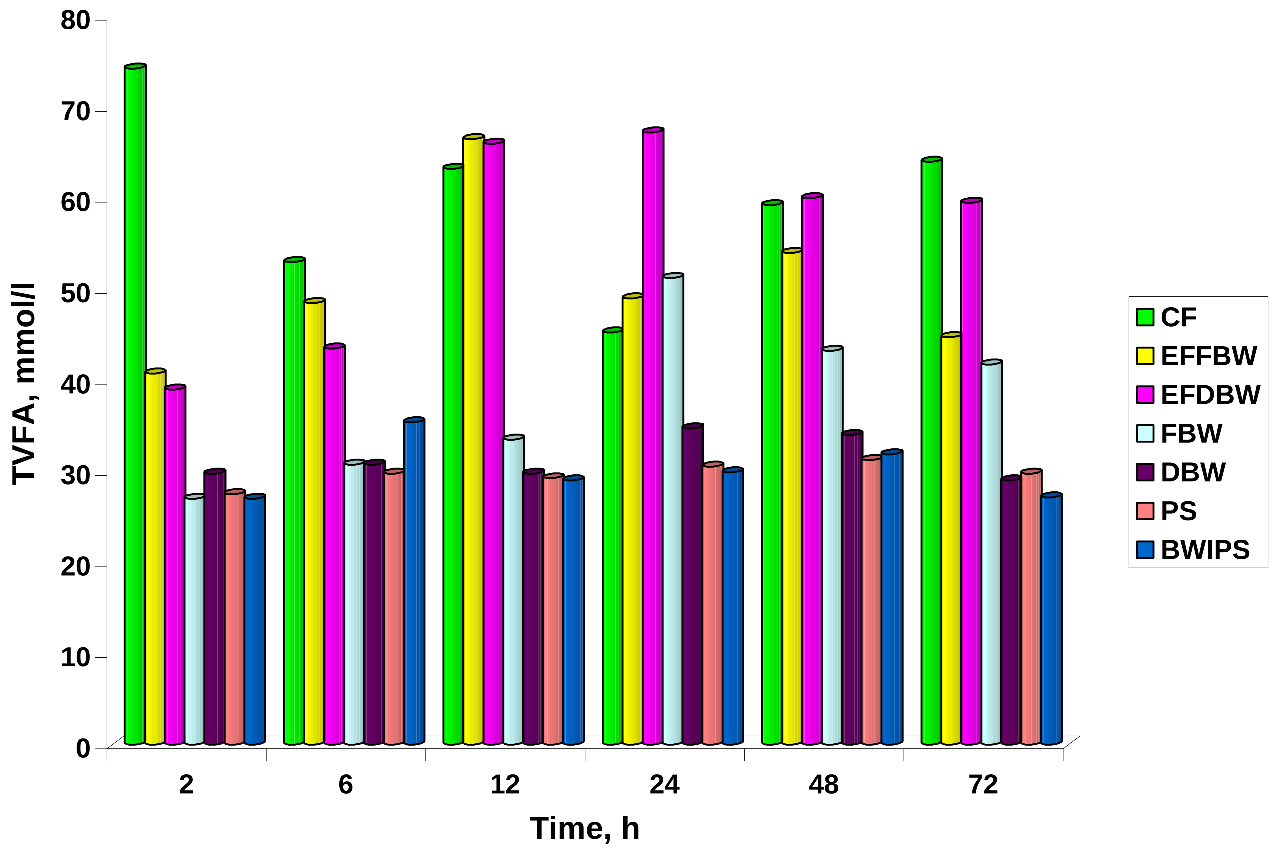


Fig. 31. ***In vitro*** **ruminal total volatile fatty acid (TVFA) production of experimental feeds at different incubation periods (h) in RUSITEC, mmol/l**

Table 30. *In vitro* ruminal volatile fatty acid fractions of experimental feeds at different incubation periods (h) in RUSITEC, mmol/l

ACETATE, mmol/l						
Treatments	2h	6h	12h	24h	48h	72h
CF	84.22	62.05	250.82	149.84	39.90	43.58
EFFBW	99.83	36.51	122.25	29.58	21.28	36.52
EFDBW	82.82	19.11	130.14	349.64	26.60	42.96
FBW	106.38	175.65	93.24	113.62	20.54	25.79
DBW	83.26	22.97	61.24	59.37	17.51	14.61
PS	50.30	51.27	94.54	56.13	5.40	18.52
BWIPS	7.47	18.30	64.49	13.37	13.43	10.50
PROPIONATE, mmol/l						
CF	56.37	68.91	197.33	103.28	33.69	38.01
EFFBW	71.31	39.68	77.68	30.14	18.06	29.94
EFDBW	69.28	27.60	111.87	266.54	26.48	31.58
FBW	79.39	132.25	68.76	99.75	29.12	44.36
DBW	51.04	10.05	34.17	43.20	22.40	20.48
PS	22.72	23.13	41.65	26.73	6.38	13.93
BWIPS	12.03	10.47	30.54	13.34	18.25	8.20
ISOBUTYRATE, mmol/l						
CF	25.34	58.37	70.41	41.91	21.69	20.44
EFFBW	35.87	0.00	33.63	12.22	5.54	18.46
EFDBW	26.18	9.38	34.41	90.63	17.68	21.77
FBW	20.15	26.63	16.13	21.77	8.57	7.91
DBW	9.96	2.53	8.27	6.11	1.71	0.00
PS	5.60	5.27	11.71	5.33	0.00	0.00
BWIPS	31.75	2.97	9.28	2.28	3.41	3.80
BUTYRATE, mmol/l						
CF	0.00	0.00	5.87	3.90	8.58	7.76
EFFBW	2.34	21.48	3.21	0.00	4.20	6.51
EFDBW	0.00	0.00	3.76	7.74	4.13	3.38
FBW	3.96	8.47	4.58	5.92	5.09	3.19
DBW	3.02	0.00	0.00	1.52	1.73	0.00
PS	0.00	0.00	0.00	0.00	0.00	0.00
BWIPS	15.50	0.00	0.00	0.00	0.00	0.30
VALERATE, mmol/l						
CF	0.00	3.16	20.89	10.59	6.20	6.53
EFFBW	7.95	2.20	8.25	3.09	1.94	5.64
EFDBW	5.35	1.13	9.84	26.48	5.00	4.49
FBW	3.93	11.78	4.88	7.51	1.81	3.91
DBW	2.81	0.00	2.46	1.42	0.08	0.00
PS	0.00	0.00	0.00	0.00	0.00	0.00
BWIPS	0.00	0.00	1.69	0.00	0.00	0.00

CF- Control feed; EFFBW – Experimental feed with fresh brewery waste (25%)
EFDBW - Experimental feed with dried brewery waste (25%);
FBW - Brewery waste (Fresh); DBW - Brewery waste (Dried); PS – Paddy straw;
BWIPS – Brewery waste incorporated paddy straw

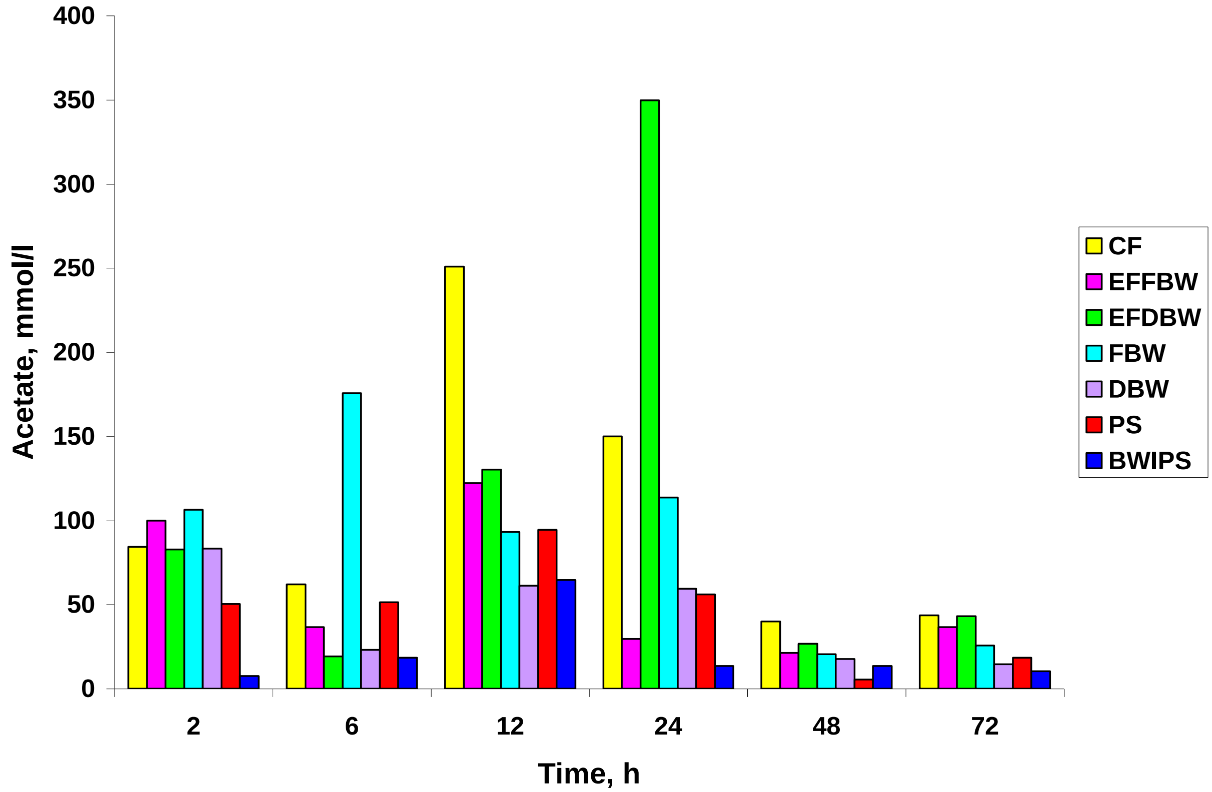


Fig. 32. ***In vitro*** **ruminal acetate concentration of experimental feeds at different incubation periods (h) in RUSITEC, mmol/l**

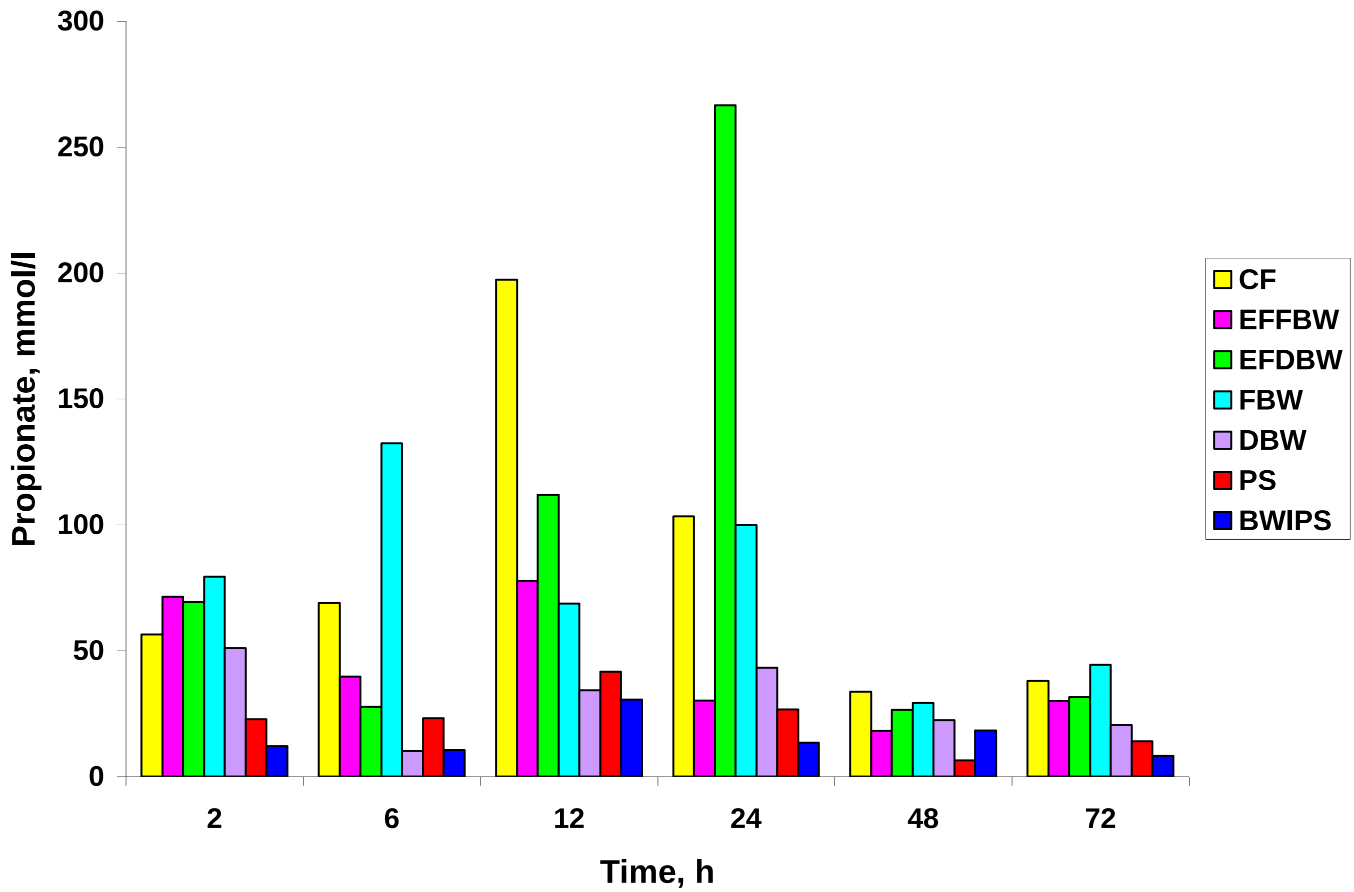


Fig. 33. ***In vitro*** **ruminal propionate concentration of experimental feeds at different incubation periods (h) in RUSITEC, mmol/l**

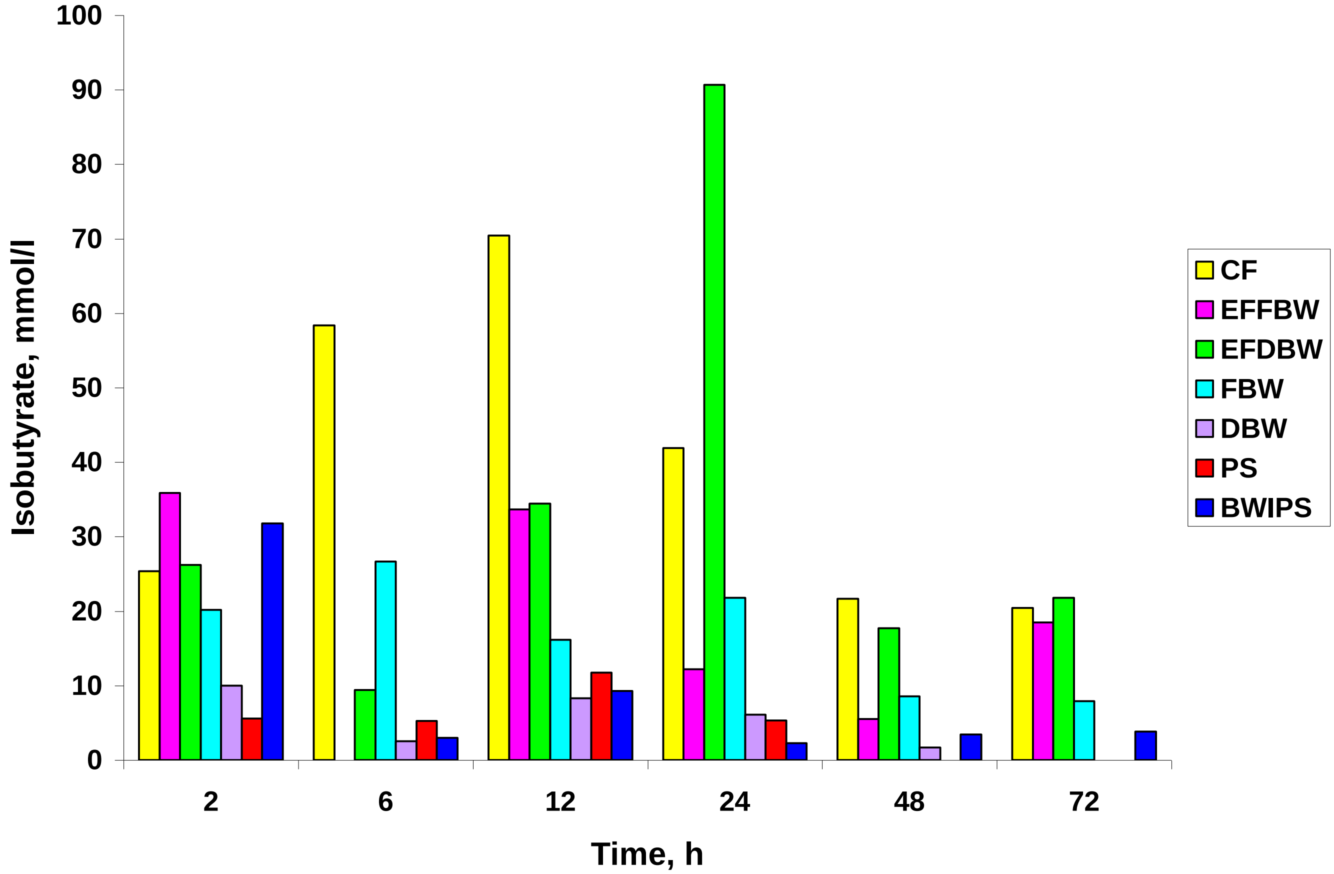


Fig. 34. *In vitro* ruminal isobutyrate concentration of experimental feeds at different incubation periods (h) in RUSITEC, mmol/l

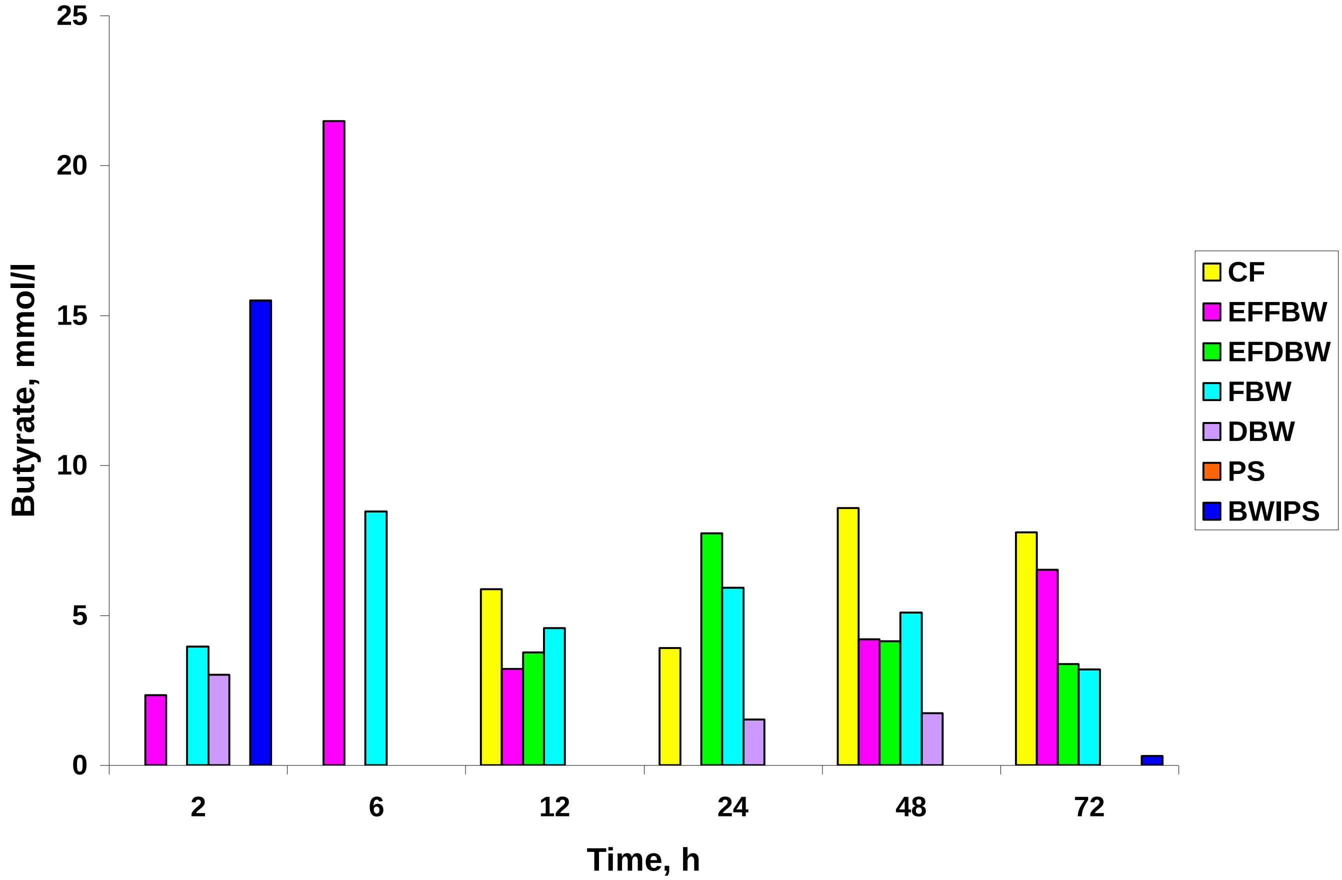


Fig. 35. *In vitro* ruminal butyrate concentration of experimental feeds at different incubation periods (h) in RUSITEC, mmol/l

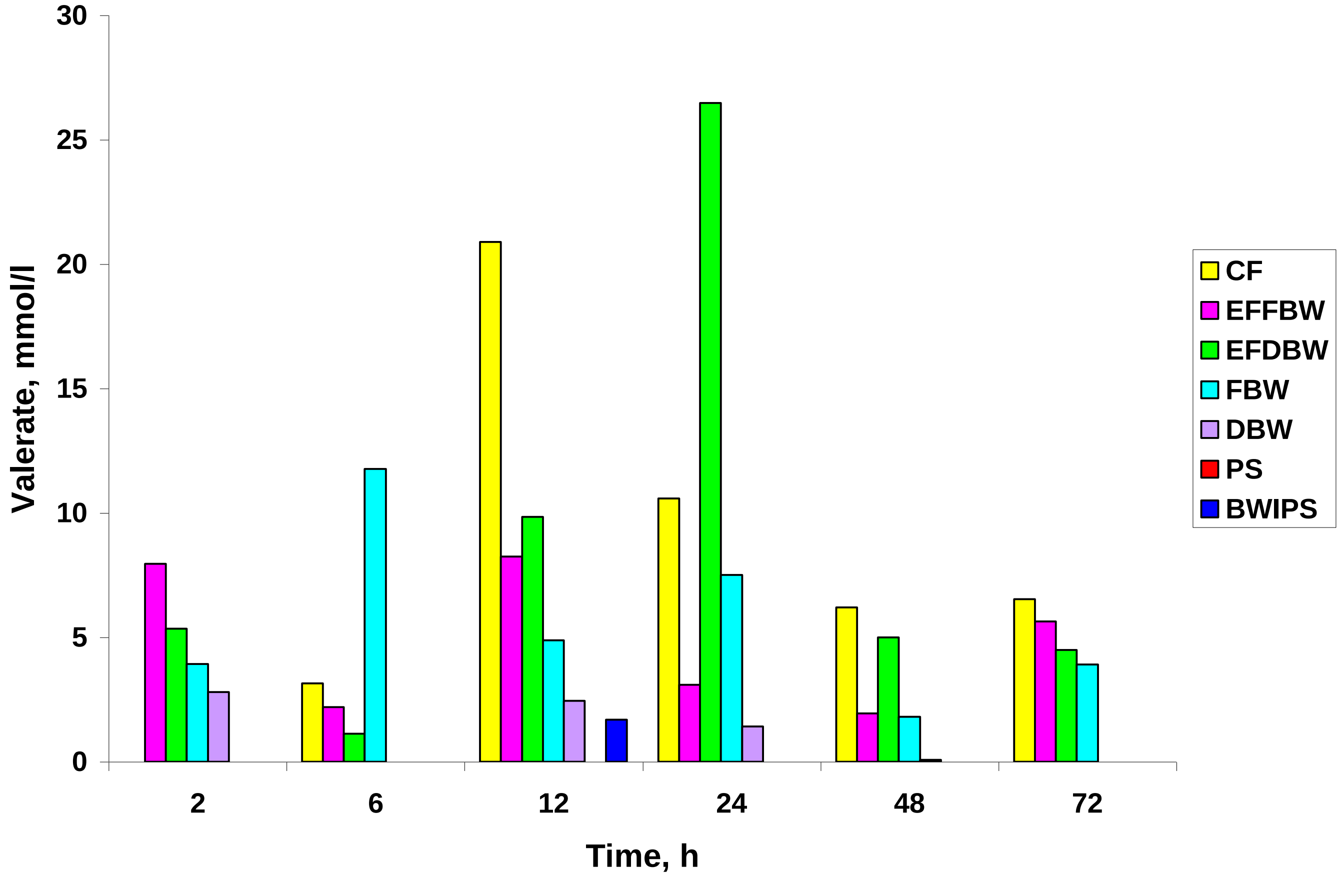


Fig. 36. *In vitro* ruminal valerate concentration of experimental feeds at different incubation periods (h) in RUSITEC, mmol/l

DISCUSSION

5. DISCUSSION

The results obtained in the present study are discussed under the following headings.

5.1 ANIMAL EXPERIMENT

5.1.1 **Chemical Composition**

5.1.1.1 Proximate Composition

5.1.1.1.1 Paddy Straw

The crude protein content of paddy straw used in this study was 4.42 ± 0.18 per cent (Table 3). Ram *et al.* (1985), Reddy and Reddy (1989), Srinivas and Gupta (1991), Keir *et al.* (1997), Srinivas and Singh (1998), Mishra *et al.* (2000) and Ally (2003) also reported similar CP content ranging from 3.82 to 5.10 per cent for paddy straw, but Reddy (1997), Dohnani *et al.* (2001), Nguyen and Uden (2001), Garg *et al.* (2002) and Nader and Robinson (2008) reported higher CP values between 5.4 and 6.1 per cent for paddy straw whereas Ramanathan (1979) and Borah *et al.* (1988) reported lower CP values of 3.10 and 3.25 per cent, respectively for paddy straw than that obtained in the present study.

The crude fibre (CF) content of paddy straw was 34.19 ± 0.61 per cent. Borah *et al.* (1988) and Srinivas and Gupta (1991) reported higher CF contents of 37.99 to 39.40 per cent, respectively, whereas Ramanathan (1979), Ram *et al.* (1985) and Reddy and Reddy (1989) reported lower CF values of 26.50, 27.20 and 23.17 per cent, respectively, for paddy straw.

The total ash (TA) content of paddy straw was 10.64 ± 0.25 per cent. Ramanathan (1979) and Nguyen and Uden (2001) also reported similar TA values for paddy straw. However, very high ash values ranging from 14.5 to 18.35 per cent were reported by Ram *et al.* (1985), Borah *et al.* (1988), Reddy and Reddy (1989), Srinivas and Gupta (1991), Krishnamoorthy *et al.* (1995), Mishra *et al.* (2000), Garg *et al.* (2002), Singh *et al.* (2002), Joseph (2005), Augustine (2008) and Nader and Robinson (2008). The acid insoluble ash (AIA) content of paddy straw was 8.76 ± 0.20 per cent.

The ether extract (EE) content of paddy straw obtained in the present investigation was 0.86 ± 0.05 per cent. Krishnamoorthy *et al.* (1995) also reported similar (0.78 per cent) EE value. Higher EE values ranging from 2.1 to 3.5 per cent were reported by Ramanathan (1979), Srinivas and Gupta (1991), Ally (2003) and Nader and Robinson (2008).

5.1.1.1.2 Brewery Waste

The DM content of brewery waste and brewery waste incorporated paddy straw was 29.15 and 78.11 per cent, respectively (Table 3). This is in agreement with Murdock *et al.* (1981), Davis *et al.* (1983) and Dong and Ogle (2003) who reported DM contents ranging from 26 to 31 per cent for wet brewer's grain. Dhiman *et al.* (2003) reported a DM value of 33.6 per cent for wet brewer's grain, while Rogers *et al.* (1986) and Belibasakis and Tsirgogianni (1996) reported lower DM values of 18.6 and 19.3 per cent, respectively.

The CP content of brewery waste and brewery waste incorporated paddy straw was 24.34 ± 0.60 and 9.41 ± 0.44 per cent, respectively. Murdock *et al.* (1981), Devendra (1992), Davis *et al.* (1983), Rogers *et al.* (1986), Belibasakis and Tirgogianni (1996), Dhiman *et al.* (2003) and Dong and Ogle (2003) also reported similar CP values ranging from 23.4 to 27.4 per cent. A higher (30.1 per cent) and lower (18.5 per cent) CP values were reported by Crickenberger and Johnson (1982) and Hayashi *et al.* (2005) respectively.

The EE content was 5.19 ± 0.18 per cent and it was found lower than the value of 6.9 and 10.6 per cent reported by Batajoo and Shaver (1998) and Dong and Ogle (2003), respectively.

The TA content of brewery waste was 5.76 ± 0.14 per cent and it was found higher than the values (3 to 5 per cent) reported by Dong and Ogle (2003).

The NFE content of brewery waste was 45.07 ± 0.79 per cent. Dong and Ogle (2003) also reported similar NFE values ranging from 41.2 to 48.1 per cent.

The acid insoluble ash content was 4.42 ± 0.15 per cent. The gross energy content of brewery waste was 3543.52 ± 46.33 kcal/kg.

5.1.1.2 Fibre Fractions

The fibre fractions of paddy straw, brewery waste and brewery waste incorporated paddy straw are presented in Table 4.

5.1.1.2.1 Paddy Straw

The NDF, ADF, hemicellulose, cellulose and lignin content of paddy straw was 73.16 ± 1.33, 48.57 ± 0.87, 24.59 ± 0.50, 34.56 ± 0.52 and 5.25 ±0.27 per cent, respectively and that of brewery waste was 54.64 ± 0.62, 24.68 ± 0.92, 29.96 ± 1.03, 13.14 ± 0.35 and 7.12 ± 0.26 per cent, respectively on DM basis.

The results indicated that the NDF and ADF values of paddy straw were higher than those reported by Rangnekar *et al.* (1982), Rai and Mudgal (1987), Rai *et al.* (1989), Reddy *et al.* (1993), Bae *et al.* (1997), Reddy (1997), Mishra *et al.* (2000), Dohnani *et al.* (2001), Nguyen and Uden (2001), Singh *et al.* (2001), Sohane and Singh (2001), Singh *et al.* (2002), Ally (2003), Joseph (2005) and Nader and Robinson (2008). Ramanathan (1979) reported much higher NDF value of 84.2 per cent and Gangwar and Sharma (2001) reported higher ADF value of 53.8 per cent for paddy straw. However, Cann *et al.* (1991) and Adeloye (2001) reported lower NDF and ADF value (66.6 and 39.07 per cent, respectively) than that of the present study.

Paddy straw had higher hemicellulose, cellulose and lignin content than those reported by Rai and Mudgal (1987), Rai *et al.* (1989), Reddy *et al.* (1993), Bae *et al.* (1997), Reddy (1997) , Mishra *et al.* (2000), Nguyen and Uden (2001), Singh *et al.* (2001), Sohane and Singh (2001), Singh *et al.* (2002) and Nader and Robinson (2008). However, Adeloye (2001) reported much higher hemicellulose value of 41.64 per cent and Gangwar and Sharma (2001) reported lower hemicellulose value of 16.6 per cent and Rangnekar *et al.* (1982) reported higher lignin value of 11.2 per cent, while Garg *et al.* (2002) reported lower lignin value of 2.70 per cent for paddy straw.

In the present study, paddy straw contained high acid insoluble ash (8.76 per cent), but the lignin content was lower (5.25 per cent). Rangnekar *et al.* (1982)

and Reddy *et al.* (1993) have also reported similar findings in paddy straw. It might be possibly due to the ash especially silica incrustation which may act like lignin components by blocking the cell wall structure of paddy straw and rendering it impermeable (Vansoest and Jones, 1968). Differences in the chemical composition of paddy straw obtained in present study may be attributed to the differences in the variety of paddy straw.

5.1.1.2.2 Brewery Waste

The NDF content of brewery waste and brewery waste incorporated paddy straw was 54.64 ± 0.62 and 68.53 ± 0.80 per cent, respectively (Table 4). Murdock *et al.* (1981), Depeters *et al.* (1997), Batajoo and Shaver (1998) and Dong and Ogle (2003) also reported similar NDF values ranging from 50 to 55 per cent for brewer's grain. Higher NDF value of 70 per cent was reported by Dhiman *et al.* (2003). On contrary, Belibasakis and Tsirgogianni (1996) and Hayashi *et al.* (2005) reported lower NDF values between 40 to 50 per cent in brewery waste.

The ADF content of brewery waste and brewery waste incorporated paddy straw was 24.68 ± 0.92 and 42.60 ± 0.84 per cent, respectively. The ADF content of brewery waste obtained in the present study is higher when compared to an average of 20 per cent as reported by Murdock *et al.* (1981), Davis *et al.* (1983), Rogers *et al.* (1986) and Depeters *et al.* (1997). However, Dhiman *et al.* (2003) reported higher ADF value of 27.7 per cent and Dong and Ogle (2003) reported lower value of 17.5 per cent in brewery waste.

The hemicellulose and cellulose content of brewery waste were 29.96 ± 1.03 and 13.14 ± 0.35 per cent, respectively and that of brewery waste incorporated paddy straw was 25.93 ± 0.56 and 29.21 ± 0.56 per cent, respectively in the present study. Valverde (1994) reported a higher hemicellulose content of 39 per cent for brewer's grain.

The lignin content of brewery waste and brewery waste incorporated paddy straw was 7.12 ± 0.26 and 5.72 ± 0.28 per cent, respectively. The results indicated that the brewery waste had moderately high lignin (7.12 ± 0.26 per cent) content when compared with the value of 4 to 5 per cent as reported by Murdock *et al.*

(1981) and Depeters *et al.* (1997). The differences in the chemical composition and fibre fractions of brewery waste observed in the present study may be attributed to the nature of grain used for beer making, differences in the manufacturing process and differences in the analytical techniques employed.

5.1.1.3 Mineral Composition

Data on the mineral composition of paddy straw, brewery waste and brewery waste incorporated paddy straw are presented in Table 5.

5.1.1.3.1 Paddy Straw

The calcium, phosphorus and magnesium content of paddy straw were 0.28 ± 0.02, 0.15 ± 0.02 and 0.14 ± 0.01 per cent, respectively on DM basis. The micro minerals such as copper, zinc, iron and manganese were 9.89 ± 0.98, 8.21 ± 3.30, 230.17 ± 10.78 and 88.65 ± 6.71 ppm, respectively.

The calcium, phosphorus and magnesium values obtained in this study are in agreement with those reported by Ranjhan (1993), Singh *et al.* (1995), Banerjee (1998), Ramana *et al.* (2000) and Nader and Robinson (2008).

The copper content of paddy straw obtained in this study is in agreement with the report of Ranjhan (1993), Banerjee (1998), Ramana *et al.* (2000) and Nader and Robinson (2008). The zinc content of paddy straw obtained in the present study was higher than that reported by Ramana *et al.* (2000). The iron content of paddy straw was in agreement with the values of 190 to 250 ppm as reported by Ranjhan (1993), Ramana *et al.* (2000) and Nader and Robinson (2008). The manganese content of paddy straw also lies within the range of 55 to 110 ppm as reported by Ranjhan (1993) and Banerjee (1998).

5.1.1.3.2 Brewery Waste

The calcium, phosphorus and magnesium content of brewery waste were 0.32 ± 0.03, 0.60 ± 0.02 and 0.16 ± 0.02 per cent, respectively on DM basis. The levels of copper, zinc, iron and manganese were 14.00 ± 1.24, 35.00± 0.02, 112.00 ± 7.85 and 21.00 ± 1.82 ppm, respectively, on DM basis.

Similar calcium and phosphorus values were reported by Crampton and Harris (1969), Titus and Fritz (1971), Sullivan *et al.* (1978), Murdock *et al.* (1981), Scott *et al.* (1982), Crickenberger and Johnson (1982) and Dong and Ogle (2003). However, lower calcium value of 0.16 per cent was reported by Gohl (1981) for brewery waste.

The magnesium content of brewery waste observed in the present study was comparatively lower than the value of 0.26 per cent reported by Dhiman *et al.* (2003). Dong and Ogle (2003) reported higher magnesium value of 0.54 per cent for brewer's grain.

Dhiman *et al.* (2003) also reported similar copper and iron content for brewer's grain as that obtained in the present experiment. Lower copper value of 7.7 ppm was reported by Dong and Ogle (2003). Higher iron content of 330 ppm for brewer's grain reported by Dong and Ogle (2003). The zinc content of brewery waste observed in this study was comparatively lower than the value of 80 to 85 ppm as reported by Dhiman *et al.* (2003). The manganese content (21 ppm) obtained in this experiment was also comparatively lower than the values of 30 to 50 ppm reported by Dong and Ogle (2003) and Dhiman *et al.* (2003).

The variations in the concentrations of both major and trace elements in brewery waste may be attributed to the possible differences in the concentration of these minerals in water, and the ingredients used for beer making and also can be due to the contamination from the walls of fermentation vat.

All breweries use malt (germinated form of barley) as the main ingredient for their brewing processes. Other grains such as maize and rice are also added along with barley in different proportions. The variation in the nutritive value of the brewery waste depends upon the type of grains used as raw material, their percentage of inclusion and the type of channels they pass through. Apart from the above mentioned reasons, variations in the nutritive value of the grains used, period of fermentation, strain variation, processing techniques and analytical procedures followed might have contributed for their variations in the proximate composition, fibre fractions and mineral composition between different investigators.

5.1.2 Body Weight

Data on the body weight of the experimental animals at the beginning (0 day) and end (60^{th} day) of the feeding trial, fed with the three dietary treatments T1, T2 and T3 are listed in Table 6 and graphically represented in Fig. 1.

The average body weight of the animals at the beginning of the feeding trial was 358.17 ± 21.15, 366.83 ± 15.37 and 336.00 ± 8.05 kg, respectively, for the three treatments T1, T2 and T3. The average body weight of the animals at the end of the feeding trial was 358.00 ± 20.78, 389.50 ± 16.57 and 354.50 ± 7.53 kg, respectively under the three treatments T1, T2 and T3. The animals of the three treatment groups such as T1, T2 and T3 showed 0, 6.0 and 5.5 per cent increase in body weight from their initial body weight.

The data on average body weight of animals at 60^{th} day of the feeding trial did not show any significant difference ($P>0.05$) among the treatment groups. This is in agreement with Chioua *et al.* (1998) who observed no significant difference in live weight of lactating dairy cows fed with wet brewer’s grain. Research workers have reported that dairy cows lose body weight in early lactation, as they will be in negative energy balance (Clark and Davis, 1980; Roffler and Thacker, 1983; Blauweikel and Kincaid, 1986 and Howard *et al.*, 1987).

Crickenberger and Johnson (1982) reported that feeding of wet brewer’s grain (33.8 per cent of diet DM) significantly increased the final body weight (at the end of 112 day) in lactating cows. Similarly, Trach (2003a) reported significant improvement in the average daily gain of growing beef bulls fed with wet brewer’s grain in both untreated and treated (3 per cent lime + 2 per cent urea) rice straw when compared to those with non supplemented untreated and treated rice straw.

Similarly Murdock *et al.* (1981) reported wet brewer’s grain increased the body weight by 7 per cent in early lactating dairy cows, but the difference was not significant. Similarly, a 3 per cent increase in body weight was reported by McCarthy *et al.* (1990) in growing lambs fed with diets containing brewer's grain.

Davis *et al.* (1983) reported that feeding of pressed brewer's grain (30 and 40 per cent DM) decreased the average body weight of lactating dairy cows.

5.1.3 Dry Matter Intake (DMI)

The average daily dry matter intake (DMI) of animals maintained on three dietary treatments T1, T2 and T3 at weekly intervals is presented in Table 7 and graphically represented in Fig. 2. The average DMI was numerically higher for those fed T2 (12.11 kg) and T3 (12.10 kg) when compared with those fed control diet (11.64 per cent). However, there were no significant differences (P>0.05) among DMI of cows received the three dietary treatments T1, T2 and T3. All the diets were consumed readily and feed refusal was almost nil throughout the experimental period.

The average DMI per 100 kg body weight of animals maintained on three dietary treatments T1, T2 and T3 was also similar (3.25, 3.11 and 3.41 per cent, respectively, Table 15). Similarly, the DMI per kg metabolic body weight of three treatment groups T1, T2 and T3 were also similar (14.14, 13.81 and 14.81 respectively, Table 15). Palmquist and Conrad (1982) reported that DMI of 13.0 and 15.5 kg per day respectively in Jersey and Holstein cows fed with distillers dried grain with solubles.

The observation made in the present study is in agreement with the reports of Murdock *et al.* (1981) in which different levels of wet brewer's grain (15 and 30 per cent of diet DM) replaced soyabean meal and barley in alfalfa hay and corn silage based diet did not affect DMI in lactating dairy cows. Similarly, no significant difference in DMI was observed in heifers and cows fed with diets containing wet brewer's grain and distiller's grains at levels of 10 to 30 per cent of dietary DM (Crickenberger and Johnson, 1982; Hoffman and Armentano, 1988; West *et al.*, 1994, Belibasakis and Tsirgogianni, 1996; Nichols *et al.*, 1998; Liu *et al.*, 2000; Dhiman *et al.*, 2003; Leonardi *et al.*, 2005 and Anderson *et al.*, 2006). Trach (2003a) also observed that DMI was not affected in growing beef bulls fed with diets containing rice straw supplemented with wet brewer's grain. However, he reported significant improvement in organic matter intake (OMI) of growing beef bulls fed with rice straw based diet with supplementation of wet brewer's grain (1.98 per cent live weight) over non-supplemented control group (1.84 per cent live weight).

In contrast, Davis *et al.* (1983) reported that when pressed brewer's grain was incorporated at 20, 30 and 40 per cent, it significantly reduced the DMI (18.2, 17.1 and 14.8 kg/day, respectively) compared to those with corn silage based control diet (19.7 kg/day). Similarly, significant reduction was observed in DMI of cows fed with diets containing distiller's grain at 20 to 30 per cent DM (Palmquist and Conrad, 1982; Lahr *et al.*, 1983; Schingoethe *et al.*, 1999 and Hippen *et al.*, 2003).

The rumen undegradable protein (RUP) content of brewer's grain and distiller's grain was around 45 to 55 per cent of CP (Shaver, 1989; Belibasakis and Tsirgogianni, 1996). Higher RUP of 71 per cent was reported by Dhiman *et al.* (2003). Kalbande and Thomas (1999) reported that increasing the level of RUP of concentrate mixture from 30 to 63 per cent increased DMI in crossbred cows maintained on low quality roughage and producing around 10 kg milk per day. Kalbande (1995) found increased DMI of cows with increasing UDP levels in concentrate mixtures containing RDP to UDP ratio of 40:60, 55:45 and 65:35.

5.1.4 Milk Production

The average daily milk production at first week of the experiment was 8.62, 10.25 and 11.28 kg, respectively for T1, T2 and T3. Similarly, the average daily milk production was 8.37, 10.39 and 11.35 kg for the second week, 8.35, 10.43 and 11.48 kg for the third week, 7.86, 10.29 and 11.25 kg for the fourth week, 7.81, 9.58 and 10.74 kg for the fifth week, 7.76, 9.77 and 10.54 kg for the sixth week, 7.97, 9.95 and 11.23 kg for the seventh week and 7.67, 10.37 and 10.93 kg for the eighth week, respectively for T1, T2 and T3 (Table 8 and Fig.3).

The overall mean of daily milk yield of experimental animals maintained on dietary treatments T1, T2 and T3 were 8.03 ± 0.27, 10.14 ± 1.07 and 11.09 ±

0.56 kg, respectively. The total milk production in 60 days by the animals maintained on three dietary treatments T1, T2 and T3 was 2889, 3651 and 3591 kg, respectively (Table 15). The total milk production in the brewery waste supplemented group (T2) was the highest followed by brewery waste incorporated paddy straw fed group (T3) among the three groups.

It was observed that milk production was significantly improved ($P<0.05$) in the brewery waste supplemented (T2) and brewery waste incorporated paddy straw (T3) fed groups when compared with those cows fed control diet (T1). The milk production was significantly increased ($P<0.05$) from fourth week onwards in brewery waste fed groups (T2 and T3) when compared with control (T1), and maintained the production throughout the experimental period.

Conrad and Rogers (1977) also reported significant improvement in milk production when cows fed with rations containing wet brewer's grain at 20 per cent DM. Similarly, inclusion of wet brewer's grain at 20 to 30 per cent of DM to the maize silage based diets significantly improved the milk production in lactating dairy cows as reported by Polan *et al.* (1985), Hoffman and Armentano (1988) and Belibasakis and Tsirgogianni (1996). An increase in milk production due to inclusion of either wet or dried distiller's grain to corn silage and alfalfa based diets in lactating dairy animals were also reported by Owen and Larson (1991), Powers *et al.* (1995), Nichols *et al.* (1998) and Anderson *et al.* (2006).

On contrary to the findings of the present study, Davis *et al.* (1983) reported reduction in milk yield of lactating dairy cows when fed with diets containing 20, 30 and 40 per cent of pressed brewer's grain. Similarly, Chioua *et al.* (1998) and Hippen *et al.* (2003) also observed reduced milk production in dairy cows fed with total mixed ration containing wet brewer's grain and wet distiller's grain at 30 per cent DM, respectively.

No significant difference in milk production of lactating dairy cows fed with diets containing wet brewer's grain and distiller's grain has been reported by various scientists (Murdock *et al.*, 1981; Hoffman and Armentano, 1988; West *et* *al.*, 1994; Al-Suwaiegh *et al.*, 2002; Dhiman *et al.*, 2003; Hippen *et al.*, 2003; Kalscheur *et al.*, 2004 and Miyazawa *et al.*, 2007).

Bypass values of the protein of wet and dry distiller's grain was 47 and 54 per cent, respectively, suggesting that drying increases the bypass value of distiller's grains (Firkins *et al.*, 1984). Santos *et al.* (1984) and Satter and Stehr (1984) reported that approximately 50 to 55 per cent of dried distiller's grain protein was undegraded in the rumen. Rumen undegradable protein content of brewer's grain and distiller's grain was around 45 to 55 per cent of CP (Shaver, 1989; Belibasakis and Tsirgogianni, 1996). Higher RUP value of 71 per cent was reported by Dhiman *et al.* (2003). The use of high RUP in diets (brewery waste) fed to dairy cows with high protein requirements can improve the aminoacid supply to the animal provided that enough degradable protein is included in the diet to maximize microbial protein (Stern *et al.*, 2006). McGuffey *et al.* (1990) reported that feeding 14 and 17 per cent CP rations with high undegradable intake protein (UIP) increased milk yield by 1.7 and 2.1 kg per day, respectively. Kalbande and Thomas (1999) reported that average daily milk yield increased in early lactating crossbred cows as RUP in the concentrate mixture increased from 38 to 63 per cent. The higher milk production obtained from animals in brewery waste fed groups (T2 and T3) of the present experiment might be due to the higher RUP in brewery waste.

5.1.5 Milk Composition

The milk collected fortnightly from the experimental animals was used for the estimation of milk constituents. The values for the milk components such as total solids, milk fat, solids not fat (SNF), milk protein and milk urea nitrogen (MUN) are given in Table 9 and graphically represented in Fig. 4, 5, 6, 7 and 8, respectively.

5.1.5.1 Total Solids

The average total solids of milk at beginning of the experiment was 13.06, 12.27 and 12.57 per cent, respectively for T1, T2 and T3. Similarly, the average total solids of milk were 12.55, 11.74 and 11.47 per cent at 15th day, 10.92, 11.24

and 10.72 per cent at 30th day and 12.06, 11.32 and 11.48 per cent at 45th day respectively, for T1, T2 and T3 (Table 9 and Fig. 4).

The average total solids of milk obtained at the 60th day of the present investigation were 12.13, 12.27 and 11.43 per cent for animals maintained on the three dietary treatments T1, T2 and T3 respectively. There was no significant difference (P>0.05) in total solids at different periods (0, 15, 30, 45 and 60 days) among the three treatment groups.

Johnson *et al.* (1987) also observed no significant difference in milk total solids content of dairy cows fed with diets containing wet brewer's grain (25 per cent DM), which is in agreement with the results obtained in the present study. Hoffman and Armentano (1988) reported that milk composition was not affected by feeding the dairy cows with diets containing either wet or dried brewer's grain. Similar observations on the lack of significant effect on the milk components when cows fed with diets containing wet brewer's grains were made by Chioua *et al.* (1998) and Miyazawa *et al.* (2007).

However, Belibasakis and Tsirgogianni (1996) reported significant improvement in milk total solids content of dairy cows fed with diets containing wet brewer's grain (12.89 per cent) compared to those of maize silage and ground maize based control diet fed animals (12.44 per cent).

On the contrary, Chioua *et al.* (1998) reported lower milk total solids content in Holstein cows fed with basal diet containing wet brewer's grain (10 per cent DM) and fresh bean curd pomace compared to soyabean meal based control diet. Ally (2003) reported a reduction in milk total solids content, when RUP level in the ration was increased from 26.8 to 42.9 per cent in dairy cows.

5.1.5.2 Milk Fat

The average milk fat percentage at beginning of the experiment was 3.62, 3.55 and 3.66 per cent, respectively for the animals maintained on three dietary treatments T1, T2 and T3. Similarly, the average fat percentage of milk was 3.43, 3.08 and 3.41 per cent at 15th day, 3.01, 3.27 and 2.96 per cent at 30th day, 3.70, 3.27 and 3.18 per cent at 45th day and 3.36, 3.58 and 3.22 per cent at 60th day, respectively, for T1, T2 and T3 (Table 9 and Fig. 5).

There was no significant difference (P>0.05) among three treatment groups at different fortnights in milk fat content.

Palmquist and Conrad (1982) observed milk fat content of 4.80 and 3.69 per cent, respectively, in Jersey and Holstein cows fed with diets containing distiller's dried grain with solubles. Inclusion of brewer's grain either in the wet or dried form in the feed had no effect on milk fat content (Murdock *et al.*, 1981; Seymour and Polan, 1986; Johnson *et al.*, 1987; Chioua *et al.*, 1998; Hoffman and Armentano, 1988 and Dhiman *et al.*, 2003), which is in agreement with the present study. Similarly, Al-Suwaiegh *et al.* (2002) and Anderson *et al.* (2006) also observed no significant difference in the milk fat content of dairy cows which were fed with alfalfa based diets containing dried distiller's grains with solubles (20 to 25 per cent DM).

However, the findings of the present study are in disagreement with the observation of Davis *et al.* (1983) in which milk fat content of lactating dairy cows was significantly increased with diets containing 20, 30 and 40 per cent pressed brewer's grain compared to those fed with soyabean meal based control diet. Belibasakis and Tsirgogianni (1996) also observed increased milk fat content in dairy cows fed with diets containing wet brewer's grain compared to those fed with maize silage and ground maize based control diet. Similarly, Anderson *et al.* (2006) also found an increase in the milk fat content of dairy cows fed with alfalfa based diets containing wet distiller's grain with solubles (20 per cent DM) compared to those with dried distiller's grains with solubles.

5.1.5.3 Solids Not Fat (SNF)

The average percentage of solids not fat (SNF) of milk at beginning of the experiment was 9.44, 8.72 and 8.92 per cent, respectively for the experimental animals maintained on three dietary treatments T1, T2 and T3. Similarly, the average SNF percentage of milk was 9.12, 8.65 and 8.06 per cent at 15th day, 7.92, 7.97 and 7.76 per cent at 30th day, 8.36, 8.05 and 8.30 per cent at 45th day and 8.77, 8.69 and 8.21 per cent at 60th day, respectively, for T1, T2 and T3 (Table 9 and Fig. 6).

There was no significant difference (P>0.05) in milk SNF percentage among the three treatment groups. This is in agreement with Belibasakis and Tsirgogianni (1996) who observed no change in milk SNF of dairy cows fed with diets containing wet brewer's grain compared to maize silage and ground maize based control diet. Dhiman *et al.* (2003) also found that there was no change in SNF levels in milk of dairy cows fed with diets containing either wet or dried brewer's grain. However, Ally (2003) reported a decrease in milk SNF content in dairy cows fed with rations containing RUP level of 42.9 per cent, compared to those fed 26.8 per cent RUP.

5.1.5.4 Milk Protein

The average percentage of milk protein at beginning of the experiment was 2.75, 2.53 and 2.82 per cent, respectively for the experimental animals maintained on three dietary treatments T1, T2 and T3. Similarly, the average protein content of milk was 2.15, 2.39 and 2.24 per cent at 15th day, 2.14, 2.33 and 2.40 per cent at 30th day, 2.73, 2.81 and 2.70 per cent at 45th day and 2.86, 3.07 and 2.76 per cent at 60th day, respectively, for T1, T2 and T3 (Table 9 and Fig. 7).

There was no significant difference (P>0.05) in milk protein at 0, 15, 30 and 45 days among the three treatment groups, but significant (P<0.05) improvement in milk protein at 60th day of milk collection was observed in brewery waste supplemented group (T2) when compared with control (T1) and brewery waste incorporated paddy straw fed group (T3). As observed in the present study, Davis *et al.* (1983) found no significant difference in milk protein content of lactating dairy cows fed with diets containing 20, 30 and 40 per cent pressed brewer's grain compared to those fed with soyabean meal based control diet. Similarly, Seymour and Polan (1986), Johnson *et al.* (1987), Hoffman and Armentano (1988), Belibasakis and Tsirgogianni (1996) and Dhiman *et al.* (2003) also could not observe any significant difference in milk protein content of lactating dairy cows fed with diets containing either wet or dried brewer's grain (10 to 25 per cent DM).

A significant increase in milk protein content was observed in brewery waste supplemented group (T2) when compared to that of T1 and T3 at 60th day of milk collection in the present study. Anderson *et al* (2006) also found significant improvement in milk protein content (3.11 per cent) of dairy cows fed with alfalfa based diets containing wet distiller's grains with solubles compared to those with dried distiller's grains with solubles (3.01 per cent) which was in agreement with findings of present study. In contrast, West *et al.* (1994) and Chioua *et al.* (1998) reported significant reduction in milk protein content of lactating dairy cows fed with diets containing wet brewer's grains. Similarly, Palmquist and Conrad (1982) observed feeding of distiller's dried grain with solubles (DDGS) based diet significantly reduced milk protein content in Jersey cows (2.85 per cent) compared to those with Holstein cows (3.40 per cent). Ally (2003) reported no significant difference in milk protein content (2.88 and 2.82 per cent) in cows fed with rations containing rumen undegradable protein levels of 26.8 and 42.9 per cent, respectively.

5.1.5.5 Milk Urea Nitrogen (MUN)

The average percentage of milk urea nitrogen (MUN) concentration at beginning of the experiment was 47.20, 46.86 and 40.22 mg/100 ml, respectively for the experimental animals maintained on three dietary treatments T1, T2 and T3. Similarly, the average MUN concentration of milk was 40.32, 40.70 and 32.64 mg/100 ml at 15th day, 37.36, 37.95 and 35.09 mg/100 ml at 30th day, 40.04, 43.95 and 35.42 mg/100 ml at 45th day and 33.66, 45.87 and 35.87 mg/100 ml at 60th day, respectively, for T1, T2 and T3. The values obtained during initial, 15, 30, 45 and 60 days are presented in Table 9 and graphically represented in Fig. 8.

There was no significant difference ($P>0.05$) in MUN concentration at 0, 15, 30 and 45 days among the three treatment groups, but significant ($P<0.05$) improvement in MUN concentration at 60th day of milk collection was observed in brewery waste supplemented group (T2) when compared with control (T1) and brewery waste incorporated paddy straw fed group (T3).

As observed in the present study, Bector *et al.* (1998) reported that MUN concentration in lactating dairy cows ranged from 22.8 to 92.4 mg/100 ml. Dhiman *et al.* (2003) reported no significant difference in MUN concentration of lactating dairy cows fed with diets containing either wet or dried brewer's grain (15 per cent DM). Anderson *et al.* (2006) also reported that there was no significant difference in MUN concentration of dairy cows fed with alfalfa based diets containing wet distiller's grain with solubles compared to those fed with dried distiller's grains with solubles (20 per cent DM). Ally (2003) reported that MUN concentration was not affected when cows were fed with rations containing 26.8 and 42.9 per cent RUP, respectively.

5.1.6 Four Per cent Fat Corrected Milk (FCM) Yield and Yield of Milk Fat and Protein

The data on the yield of four per cent FCM, milk fat and milk protein are given in Table 10 and graphically represented in Fig. 9 and 10.

The mean four per cent FCM yield of animals of T1, T2 and T3 was 462.97, 536.89 and 533.68 kg, respectively. The four per cent FCM yield tended to increase in brewery waste fed groups T2 and T3, but there was no significant difference (P>0.05) among three treatment groups.

Hoffman and Armentano (1988) and Dhiman *et al.* (2003) observed no significant difference in the four per cent FCM yield of lactating dairy cows fed with diets containing either wet or dried brewer's grains (15 to 30 per cent). Augustine (2008) also reported that four per cent FCM yield of cows were not affected by protected fat supplementation in maize waste, tapioca starch waste, brewery waste based ration. However, a significant improvement in the four per cent FCM yield of lactating dairy cows fed with diets containing wet brewer's grain was observed by Belibasakis and Tsirgogianni (1996) and Chioua *et al.* (1998).

The mean milk fat yield of animals maintained on three dietary treatments T1, T2 and T3 was 17.36, 20.01 and 19.62 kg, respectively. The average yield of milk protein from animals of T1, T2 and T3 was 12.80, 15.50 and 15.45 kg, respectively. No significant difference in milk fat and milk protein yield was observed among three treatment groups.

5.1.7 Haematological and Biochemical Parameters

Data on the haematological parameters such as haemoglobin and various biochemical parameters such as plasma glucose, calcium, phosphorus and plasma urea nitrogen (PUN) levels of the blood samples of cows maintained on three dietary treatments T1, T2 and T3 collected at initial, 30th and 60th day of the feeding trial are given in Table 12 and graphically represented in Fig. 14.

All the values obtained were within the normal levels specified for cows (Kaneko and Harvey, 1997). There was no significant difference ($P>0.05$) among the three treatment groups T1, T2 and T3 in any of the parameters studied, except that of plasma urea nitrogen.

5.1.7.1 Haemoglobin

The average haemoglobin concentration at the end of the feeding trial was 8.80, 9.22 and 9.18 g/dl, respectively for the animals maintained on three dietary treatments T1, T2 and T3. There was no significant difference ($P>0.05$) in haemoglobin concentration at 0, 30th and 60th day among the three treatment groups. The values concur with those reported (8.99 to 9.73 g per cent) by Ally (2003), Joseph (2005) and Augustine (2008) in different experiments.

However, Hareesh (2007) reported higher values of 12.18 and 12.88 g per cent for different treatments than those obtained in the present study. Ramakrishna (2003) observed uniform levels of haemoglobin (9.92 to 10.59 g per cent) in Jersey cross bred cows maintained on different feeding regimens with straw based ration.

5.1.7.2 Plasma glucose

The average plasma glucose levels of experimental animals of three treatment groups T1, T2 and T3 were 51.92, 54.42 and 56.42 mg per cent, respectively on 60th day of the feeding trial. The values concur with those reported (49.93 to 59.91 mg per cent) by Joseph (2005) and Augustine (2008) in different experiments. There was no significant difference (P>0.05) among the plasma glucose levels of the three groups which was in agreement with the findings of Smith *et al.* (1978) and Palmquist and Conrad (1982). Delahoy *et al.* (2003) also reported no significant difference in plasma glucose levels when cows were fed with steam flaked corn and ground corn.

As observed in the present study, Palmquist and Conrad (1982) reported no significant improvement in the blood glucose concentration of cows fed with ration containing distiller's dried grains with solubles (48 per cent DM). Similarly, Belibasakis and Tsirgogianni (1996) also found no significant difference in blood glucose concentration of cows fed with ration containing wet brewer's grain (16 per cent DM).

Hypoglycemic condition during early lactation can be expected due to drainage of glucose for lactose synthesis. However, none of the animals showed any signs of ketosis.

5.1.7.3 Plasma Urea Nitrogen (PUN)

No significant difference (P>0.05) in PUN concentration could be seen among the three treatments at 30th day of feeding trial. The PUN levels in the brewery waste incorporated (T2) group (41.45 mg/100 ml) was significantly higher (P<0.05) than that of (T1) control (32.48 mg/100 ml) and brewery waste incorporated paddy straw fed (T3) group (30.10 mg/100 ml) on the 60th day of blood collection.

The observation made in the present study is in agreement with the reports of Belibasakis and Tsirgogianni (1996) in which supplementation of wet brewer's grains (16 per cent DM) to the dairy cows did not influence the PUN concentration (25.5 mg/100 ml) when compared with those fed with maize silage based control diet (25.3 mg/100 ml). Similarly, Huang *et al.* (1999) found no significant difference in serum urea nitrogen (SUN) when dairy cows were fed with alfalfa hay based ration containing (20 per cent DM) dried rice distiller's grain (10.58

mg/100 ml) compared to those fed with soyabean meal based control diet (10.84 mg/100 ml) at the end of 2nd month.

Murdock *et al.* (1981) also reported significant improvement in PUN concentration of lactating dairy cows fed with alfalfa hay based rations containing brewer's grain (17 mg/100 ml) compared to those fed with soyabean meal based diet (15 mg/100 ml). Similarly, McCarthy *et al.* (1990) found that wet brewer's grain significantly improved the blood urea nitrogen (BUN) concentration (19 mg/100 ml) compared to those fed with ground corn based diet (14.3 per cent) in growing lambs.

5.1.7.4 Plasma Calcium and Phosphorus

The average plasma calcium levels of the three treatment groups were 9.87, 10.12 and 10.20 mg per cent, while the plasma phosphorus levels were 6.07, 6.16 and 6.63 mg per cent, respectively, for the cows of T1, T2 and T3 on 60th day of the feeding trial. There was no significant difference ($P>0.05$) among the three treatments.

Belibasakis and Tsirgogianni (1996) found that supplementation of wet brewer's grain did not influence the plasma calcium and phosphorus (9.4 and 6.0 mg per cent, respectively) when compared to those fed with maize silage based control diet (9.7 and 6.1 mg per cent, respectively) in lactating dairy cows.

The observations of the present study are in agreement with the reports of Ally (2003), Joseph (2005) and Augustine (2008) who observed no difference in plasma calcium and phosphorus level in cross bred cows maintained on different feeding regimens with paddy straw based ration.

5.1.8 Rumen Fermentation Parameters

The average ruminal pH, ammonia nitrogen (NH_3-N) and total volatile fatty acids concentrations estimated from the rumen liquor collected at the end of feeding trial from the cows maintained on three dietary treatments are given in Table 11 and graphically represented in Fig. 11, 12 and 13.

5.1.8.1 pH

The average pH of the rumen liquor was 6.96, 6.75 and 6.87 respectively, for the three dietary treatments. There was no significant difference (P>0.05) in ruminal pH among the three treatment groups. The pH of rumen liquor in all the treatment groups of present study was within the normal levels specified for cows. A ruminal pH of 6.4 was reported by Claypool *et al.* (1980) and Forster *et al.* (1983), while Klusmeyer *et al.* (1990) and Weigel *et al.* (1997) reported ruminal pH of below six.

Similarly, Murdock *et al*. (1981) observed no significant difference in the ruminal pH of lactating dairy cows fed with rations containing wet brewer's grain. No significant difference in ruminal pH of cows fed with diets containing either wet or dried distiller's grain at 10 to 25 per cent DM was reported by Huang *et al.* (1999), Al-Suwaiegh *et al.* (2002) and Cruz *et al.* (2005).

However, Davis *et al*. (1983) observed significant increase in the pH of rumen liquor from cows fed 20, 30 and 40 per cent pressed brewer's grain supplemented diets compared to those with non supplemented diet. Similarly, McCarthy *et al.* (1990) found significant increase in rumen liquor pH of growing lambs fed with diets containing wet brewer's grain (pH, 6.03) compared to those fed ground corn control diet (pH, 5.63).

On contrary, Trach (2003b) reported significant reduction in pH of rumen liquor due to addition of 10 per cent of wet brewer's grain in fistulated animals fed with rice straw based diet. He further reported that supplementation of 10 per cent wet brewer's grain to treated rice straw (3 per cent lime + 2 per cent urea) based diet significantly reduced the pH from 6.71 to 6.34 in fistulated animals. Similarly, the ruminal NDF digestion rate and extend of distiller's grain (Table 14) were high, reflecting the high fibrolytic activity associated with ruminal pH greater than 6.2 (Grant, 1994).

5.1.8.2 Ammonia- Nitrogen (NH_3-N)

The average NH_3-N concentration was 24.88, 27.85 and 27.34 mg/100 ml of rumen liquor, respectively, for the animals maintained on three dietary treatments T1, T2 and T3 at the end of the feeding trial. There was no significant difference when analysed statistically.

The mean ammonia concentration in the rumen liquor on all diets remained above the 5 mg/100 ml suggested (Roffler and Satter, 1975) as the minimum requirement for the maintenance of rumen microbial growth.

In the current study, ruminal NH_3-N concentration was not affected by the inclusion of brewery waste in the ration. This observation is in agreement with reports of various researchers (Huang *et al.*, 1999; Schingoethe *et al.* 1999 and Cruz *et al.*, 2005) indicating no significant effect on ruminal NH_3-N concentration by including distiller's grain or wet brewer's grain (McCarthy *et al.*, 1990 and Trach, 2003b) in the diet of lactating dairy cows. However, a significant improvement in ruminal NH_3-N concentration of lactating dairy cows fed with diets containing wet brewer's grain (30 per cent DM) was reported by Murdock *et al.* (1981).

On contrary, Kleinschmit *et al.* (2006) reported significant reduction in ruminal NH_3-N concentration of lactating dairy cows fed with diets containing distiller's dried grains with solubles (20 per cent DM) compared to those fed with alfalafa hay and corn silage based control diet having. Similarly, Anderson *et al.* (2006) found significant reduction in NH_3-N concentration in rumen liquor of cows when fed with 20 per cent, compared to those with 10 per cent dried distiller's grain solubles based diet. They further observed that the cows fed with 10 per cent wet distiller's grain solubles incorporated diet had lower NH_3-N concentration than those fed with 20 per cent.

The correlations were higher between CP intake and soluble nitrogen intake and between ruminal NH_3-N and PUN concentration for animals fed the wet brewer's grain rations. These relationships reflect the higher solubility of protein in wet brewer's grain, resulting in higher ruminal NH_3-N and PUN in relation to dietary intake (Murdock *et al.* 1981). On contrary, Owen and Larson (1991) reported significant reduction in SUN concentration in lactating Holstein cows fed with diets containing distiller's dried grains with solubles (14 mg/100 ml) compared to those fed with soyabean meal based diet (18 mg/100 ml). Similarly, plasma urea nitrogen decreased in cows supplemented with steam flaked corn and ground corn (Delahoy *et al.* 2003).

5.1.8.3 Total Volatile Fatty Acids (TVFA)

The average TVFA concentrations of rumen liquor collected from animals fed with three dietary treatments T1, T2 and T3 were 101.31, 99.44 and 94.63 meq/l, respectively, at the end of feeding trial. The values were not significantly different (P>0.05) among the treatments and were within the normal range of 50 to 150 meq/l (McDonald *et al.* 1995).

The observations made in the present study are in agreement with those of Al-Suwaiegh *et al.* (2002), Cruz *et al.* (2005) and Anderson *et al.* (2006) and they did not find any significant effect on TVFA production by including either wet or dried distiller's grain at 10 to 20 per cent of DM in the diet of lactating dairy cows.

Huang *et al.* (1999) on the other hand, found significant improvement in rumen TVFA concentration of cows fed with diets containing 10 per cent rice distiller's grain (88.16 mmol/l) compared with those fed with 20 per cent inclusion (78.17 mmol/l). Similarly, Trach (2003b) reported that wet brewer's grain supplementation significantly improved the rumen TVFA concentration of oxen fed with both untreated (103 mmol/l) and treated (3 per cent lime with 2 per cent urea) rice straw (114 mmol/l) compared with those fed a diet without brewery waste (68 to 93 mmol/l). Rogers *et al.* (1986) observed that feeding of corn silage based diet containing either wet or dried brewer's grain significantly improved the rumen TVFA concentration in steers.

Reduced TVFA concentration in rumen liquor was reported by Davis *et al.* (1983) when lactating dairy cows were fed diets containing pressed brewer's grains. Similarly, Schingoethe *et al.* (1999) and Kleinschmit *et al.* (2006) also reported that feeding of distiller's grain (20 to 30 per cent DM) to lactating dairy cows significantly reduced the TVFA concentration in rumen liquor.

5.1.9 Apparent Digestibility

The chemical composition of dung of experimental animals and apparent digestibility of DM, OM, CP, CF, EE, NFE, gross energy and fibre fractions such as NDF, ADF, hemicellulose and cellulose of the three experimental rations are presented in Tables 13 and 14, respectively. The data regarding the apparent digestibility of nutrients and fibre fractions are illustrated in Fig. 15 and 16, respectively.

There was no significant difference ($P>0.05$) in apparent digestibility of DM, OM, CP, EE, NFE, ADF, cellulose and gross energy among the three treatment rations. The apparent digestibility of CF, NDF and hemicellulose was significantly higher ($P<0.05$ for CF and NDF and $P<0.01$ for hemicellulose) for T2 and T3 compared to those fed control diet.

5.1.9.1 Dry Matter

The average dry matter digestibility of the three experimental rations T1, T2 and T3 was 56.43, 57.90 and 54.94 per cent, respectively. There was no significant difference ($P>0.05$) in DM digestibility among the three experimental rations.

Firkins *et al.* (1984) observed that feeding of wet and dried distiller's grain (25 per cent DM) based diet showed similar DM digestibility (57.7 and 57.3 per cent, respectively) which was similar to those observed in the present study.

No significant effect on DM digestibility of distiller's grain based diet in lactating dairy cows was reported by Weiss *et al.* (1989) and Birkelo *et al.* (2004) which agrees well with the results obtained in the present study. Similarly, Armentano *et al.* (1986) and Rogers *et al.* (1986) reported that feeding of wet or dried brewer's grain based diet did not influence the DM digestibility in ruminants.

Higher DM digestibility of 60 to 67 per cent was reported by Firkins *et al.* (1985), Birkelo *et al.* (2004), Peter *et al.* (2000) and Harborth *et al.* (2006) of diets containing wet corn distiller's grain, dried corn distiller's grain and dried sorghum distiller's grain.

However, Shelford and Tait (1986) observed that feeding of sheep with diets containing corn distiller's grain significantly improved the DM digestibility (77.7 per cent) compared to those fed with rye distiller's grain (67.5 per cent). McCarthy *et al.* (1990) observed that DM digestibility was increased significantly in wet brewer's grain based diet (75.3 per cent) compared to those fed with alfalfa based control diet (70.0 per cent) in growing lambs.

5.1.9.2 Organic Matter

The average organic matter (OM) digestibility of the three experimental rations T1, T2 and T3 was 59.12, 60.75 and 58.34 per cent, respectively. There was no significant difference (P>0.05) in organic matter digestibility among the three treatment rations.

Merchen *et al.* (1979) found that feeding of brewer's grain did not influence OM digestibility (60.7 per cent) compared to those fed with soyabean meal based diet (60.7 per cent). The observed values in the present study are in agreement with the reports of Firkins *et al.* (1984) and Trach (2003a) with regard to organic matter digestibility while feeding distiller's grain and brewer's grain incorporated rations.

Shelford and Tait (1986) reported that corn distiller's grain significantly improved the OM digestibility compared to those fed with rye distiller's grain. Peter *et al.* (2000) found higher OM digestibility of 65 per cent in diets containing dried corn distiller's grain in lactating dairy cows. Similarly, Trach (2003a) observed a significant improvement in the OM digestibility of untreated and treated (3 per cent lime with 2 per cent urea) rice straw as a result of wet brewer's grain supplementation in growing beef bulls.

5.1.9.3 Crude Protein

The crude protein digestibility of three experimental rations T1, T2 and T3 was 60.13, 51.71 and 52.62 per cent, respectively. There was no significant difference (P>0.05) in CP digestibility among the three treatment rations, but the CP digestibility of brewery waste incorporated rations T2 and T3 were numerically lower than that of control.

Firkins *et al.* (1985) reported that there was no significant difference in CP digestibility between diets containing wet and dried distiller's grain in ruminants as observed in the present study.

The values observed in the present study are in agreement with the reports of Rogers *et al.* (1986) and Weiss *et al.* (1989), in which no significant effect in CP digestibility (49.6 and 57.6 per cent, respectively) was observed for diets containing brewer's grain and distiller's grain in ruminants. Shelford and Tait (1986) also observed no significant difference in CP digestibility of diets containing rye distiller's grain (79.6 per cent) and corn distiller's grain (81.2 per cent) in sheep.

Edionwe and Owen (1989) reported that the CP digestibility of diets containing corn distiller's grains had significantly lower (50.6 to 55.8 per cent) compared to those with soyahulls based control diet (73 per cent) in Holstein cows.

Higher CP digestibility values of 74 to 80 per cent of diets containing wet corn distiller's grain were reported by Lodge *et al.* (1996) and Birkelo *et al.* (2004) and for dried corn and sorghum distiller's grain incorporated ration by Lodge *et al.* (1996), Lodge *et al.* (1997a) and Fastinger and Mahan (2006).

On contrary, Peter *et al.* (2000) observed lower CP digestibility of 41 per cent with diets containing dried corn distiller's grain in ruminants.

From the RUSITEC experiment, the effective CP degradability of paddy straw, control concentrate mix and experimental concentrate mix (containing 25 per cent brewery waste) were 28.64, 53.97 and 52.37 per cent (Table 24). The average RUP intake of the animals of three experimental groups T1, T2 and T3 were 0.68, 0.71 and 0.72 kg, respectively. As per NRC (2001), the RUP requirement in the ration of early lactating cows producing 15 kg milk is 0.50 kg per day. It shows that, the experimental rations satisfied the RUP requirement of animals used in the present study.

5.1.9.4 Crude Fibre

The crude fibre digestibility was 50.27, 57.48 and 56.34 per cent for the three treatment rations T1, T2 and T3, respectively, in the present study. It was observed that the inclusion of brewery waste in the ration T2 and T3 significantly increased ($P<0.05$) the CF digestibility compared to those fed control diet. The higher CF digestibility in T2 and T3 might be due to the presence of easily fermentable structural carbohydrates and amino acids and other growth factors for optimum growth and cellulolytic activity of rumen microbes as reported by Tamminga *et al.* (1990) and Ojowi *et al.*, (1997) and also due to the action of microbial enzymes, which had acted on β,1-4 linkages of hemicellulose (xylan) to release soluble sugars as reported by Bhat and Hazelwood (2001).

Rao *et al.* (2001) found significant improvement in the CF digestibility of diets containing yeasac (yeast culture) supplement in rams. On contrary, Peter *et al.* (2000) observed lower CF digestibility (42 per cent) of diets containing dried corn distiller's grain in lactating dairy cows.

5.1.9.5 Ether Extract

The digestibility of ether extract (EE) for the three treatment rations T1, T2 and T3 were 53.28, 51.69 and 56.63 per cent, respectively. No significant difference ($P>0.05$) in EE digestibility could be seen among the three treatments.

As observed in the present study, Edionwe and Owen (1989) observed that digestibility of EE was not significantly affected when medium yielding cows were fed with diets containing corn distiller's grain (76.9 per cent) compared with those fed soyhulls based diet (70.7 per cent). However, Birkelo *et al.* (2004) found significant improvement in EE digestibility of lactating dairy cows fed with diets containing wet corn distiller's grain (78.4 per cent) compared to those fed with corn silage and alfalfa hay based control diet (67.6 per cent).

5.1.9.6 Nitrogen Free Extract (NFE)

The NFE digestibilities were 65.50, 66.78 and 65.52 per cent, respectively for the dietary treatments T1, T2 and T3. There was no significant difference (P>0.05) in the NFE digestibility among the three treatments. Similar values were reported by Ally (2003), Hareesh (2007) and Augustine (2008) in different experiments.

5.1.9.7 Gross Energy

The gross energy (GE) digestibility was 56.16, 57.59 and 54.60 per cent, respectively for the dietary treatments T1, T2 and T3. No significant difference (P>0.05) in GE digestibility could be seen among the three treatments. Shelford and Tait (1986) observed significant improvement in energy digestibility of corn distiller's grains (78.5 per cent) compared to those fed with rye distiller's grains (62.7 per cent) in sheep. The digestible energy content of the three experimental rations T1, T2 and T3 were 2095 ± 118.90, 1929.83 ± 99.06, 1826.47 ± 133.86 kcal/kg, respectively.

5.1.9.8 Fibre Fractions

The digestibility of fibre fractions such as NDF, ADF, hemicellulose and cellulose of the three treatment rations T1, T2 and T3 are presented in Table 14 and graphically represented in Fig. 16.

5.1.9.8.1 Neutral Detergent Fibre (NDF)

The average NDF digestibility was 47.31, 54.52 and 55.63 per cent, respectively for the dietary treatments T1, T2 and T3. It was observed that, inclusion of brewery waste in the ration T2 and T3 significantly (P<0.05) increased the NDF digestibility compared to those fed control diet. The values obtained in the present study are in agreement with those obtained by Ally (2003) and Augustine (2008).

Firkins *et al.* (1984) observed that NDF digestibility was significantly higher for angus hereford steers fed wet distiller's grain (62.6 per cent) and dried distiller's grain (63.5 per cent) compared with those of steers fed wet corn gluten feed (53.8 per cent) and dried corn gluten feed (47.2 per cent). Higher NDF digestibility values (60 to 70 per cent) for diets containing both wet and dried distiller's grain have been reported (Firkins *et al.*, 1985; Ham *et al.*, 1994; Lodge *et al.*, 1996; Lodge *et al.*, 1997b; Al-Suwaiegh *et al.*, 2002 and Birkelo *et al.*, 2004).

The results obtained in the present study are in agreement with reports of Firkins *et al.* (1985); Weiss *et al.* (1989); Ham *et al.* (1994) and Birkelo *et al.* (2004) in which distiller's grain feeding significantly improved the NDF digestibility in ruminants. Similarly, McCarthy *et al.* (1990) found that feeding wet brewer's grains to the growing lambs significantly improved the NDF digestibility. On contrary, Al-Suwaiegh *et al.* (2002) observed that there was no significant difference in NDF digestibility between wet and dried distiller's grain feeding to lactating dairy cattle.

The average hemicellulose digestibility was 36.85, 44.64 and 46.17 per cent, respectively for the treatment rations T1, T2 and T3 in the presents study. It was observed that the hemicellulose digestibility of brewery waste incorporated rations T2 and T3 was significantly improved ($P<0.01$), compared to those fed with control ration (T1).

The average cellulose digestibility was 36.95, 38.65 and 40.03 per cent, respectively for the treatment rations T1, T2 and T3. There was no significant difference ($P>0.05$) in cellulose digestibility among the three treatments.

5.1.9.8.2 Acid Detergent Fibre (ADF)

The ADF digestibility obtained in the present study was 39.10, 43.53 and 40.83 per cent for the three rations T1, T2 and T3 respectively. There was no significant difference ($P>0.05$) in the ADF digestibility among the three treatment rations. The ADF digestibility values in brewery waste fed groups (T2 and T3) observed in the present study are in agreement with reports of Edionwe and Owen (1989).

Shelford and Tait (1986) reported that there was no significant difference in ADF digestibility of diets containing corn distiller's grain (51.7 per cent) and rye distiller's grain (45.9 per cent) when fed to Holstein-Friesian cows. Al-Suwaiegh *et al.* (2002) also reported that there was no significant difference in ADF digestibility between wet and dried distiller's grain feeding in lactating dairy cattle.

The ADF digestibility of diets containing either wet or dried distiller's grain was 55 to 65 per cent as reported by Al-Suwaiegh *et al.* (2002) and Birkelo *et al.* (2004) and 72 per cent as reported by Firkins *et al.* (1985) which were higher than the values obtained in the present study.

However, McCarthy *et al.* (1990) reported that wet brewer's grain feeding significantly improved the ADF digestibility in growing lambs. Birkelo *et al.* (2004) also found significant improvement of ADF digestibility in lactating dairy cows fed with diets containing wet corn distiller's grain (57.7 per cent) compared to those fed with corn silage and alfalfa hay based control diet (46.4 per cent).

Higher CF, NDF and hemicellulose digestibility was obtained for T2 and T3. This may be due to presence of easily fermentable structural carbohydrates and amino acids and other growth factors in wet brewer's grain for optimum growth and cellulolytic activity of rumen microbes. The combined action of these factors would have helped in enhancing the digestibility of CF, NDF and hemicellulose as reported by Tamminga *et al.* (1990) and Ojowi *et al.* (1997).

Bartolome *et al.* (2002) have shown that it is possible to liberate pentoses (xylose and arabinose) and hydroxy cinnamicacids (ferulic acid and p-coumaric acid) from brewer's spent grain by treatment with commercially available enzyme preparations and plant enzyme extracts. Since brewery waste is a fermented material, the microbes and microbial enzymes present in the material would have acted on the paddy straw fibre in brewery waste incorporated paddy straw (T3) fed group, thereby altering the structure of fibre in paddy straw as reported by Sim and Oh (1990).

The higher hemicellulose digestibility of brewery waste incorporated rations could be due to the action of microbial enzymes, which had acted on β,1-4 linkages of hemicellulose (xylan) to release soluble sugars as reported by Bhat and Hazelwood (2001).

5.1.10 Economics of Milk Production

Data on the feed intake, total milk production and economical assessment such as cost of feed per kg milk produced by experimental cows maintained on three dietary treatments are given in Table 15 and cost of feed per kg milk production are graphically represented in Fig.17.

The total milk produced in 60 days by the animals fed the three dietary treatments T1, T2 and T3 were 2888.50, 3651.10 and 3591.30 kg, respectively. The milk production was higher in brewery waste fed groups (T2 and T3) than those fed control diet.

The cost of concentrate mixtures per kg for T1, T2 and T3 were Rs.11.07, 10.38 and 10.38, respectively. The cost of feed per kg milk produced was Rs.10.40, 7.89 and 8.37 respectively, for the three dietary treatments T1, T2 and T3.

The cost of milk production was highest in animals maintained on control (T1) ration because of low milk production and higher cost of feed. The brewery waste fed groups (T2 and T3) had lower cost of feed per kg milk produced compared to those fed control diet (T1), due to higher milk production and comparatively low cost of feed. The cost of brewery waste incorporated feed was comparatively lower due to low cost of brewery waste (Rs.2.00 per kg on fresh basis).

Dhiman *et al.* (2003) reported that wet brewer's grain feeding was advantageous than dried brewer's grain feeding to lactating dairy cows. However, wet brewer's grains have a very short shelf life of 3 to 7 days (Rendell, 2004). In two week times, the brewery waste becomes spoiled and develops an offensive odour which will attract flies resulting in maggot infestation. Also, transportation, shipment and drying process of brewery waste is expensive because of the added water weight. If these factors are optimized, brewery waste can lower the cost of production for livestock producers.

Myer and Hall (2003) also reported that the transportation, storage and handling cost limit the use of wet brewer's grains to relatively large cattle operations located near the breweries.

An overall evaluation of the results obtained during the present investigation revealed that the inclusion of (25 per cent DM) brewery waste in paddy straw based diet in medium yielding early lactation cows significantly improved the milk production as well as the digestibility of crude fibre, neutral detergent fibre and hemicellulose. However, no significant effect of brewery waste inclusion on dry matter intake, body weight gain, four per cent fat corrected milk (FCM) yield, milk and blood composition was observed. The cost of feed per kg milk produced was also reduced by feeding the ration containing brewery waste (25 per cent DM) in early lactating crossbred cows producing about 10 kg milk per day.

Therefore, in areas that are close to a brewery it seems profitable to include brewery waste in dairy rations at the level of 25 per cent (DM basis) in concentrate mixture.

5.2 RUSITEC EXPERIMENT

The results obtained in the RUSITEC study are discussed under the following headings.

5.2.1 *In Vitro* Disappearance / Degradability Studies in RUSITEC

5.2.1.1 Dry Matter

The *in vitro* DM disappearance/degradability (IVDMD) of control feed, experimental feed with fresh brewery waste (EFFBW), experimental feed with dried brewery waste (EFDBW), fresh brewery waste (FBW), dried brewery waste (DBW), paddy straw (PS) and brewery waste incorporated paddy straw (BWIPS) at 2 hours of incubation in RUSITEC was 26.88, 25.38, 24.25, 16.66, 16.02, 15.62 and 15.73 per cent, respectively and at 72 hours of incubation was 63.78, 62.36, 60.70, 52.68, 46.76, 33.24 and 42.25 per cent, respectively (Table 16 and Fig. 18).

The DM disappearance values obtained in the present *in vitro* (RUSITEC) study (52.68 per cent) agrees with the value obtained by Orskov *et al.* (1992) in which the ruminal *in situ* disappearance of brewer's grain was 53.8 per cent. However, the IVDMD of FBW and DBW obtained in this study (52.68 and 46.76 per cent, respectively) were lower than that of Geetha (2007) for distiller's grain (68.52 per cent) in RUSITEC. Similarly, Reed *et al*. (2006) reported higher *in vitro* organic matter disappearance (IVOMD) of 79.33 per cent for corn distiller's dried grains with solubles by Tilley and Terry (1963) method than that of the present study.

The IVDMD of paddy straw at 2 hours of incubation was 15.62 per cent and it disappeared / degraded upto 30.51 and 33.24 per cent at 48 and 72 hours of incubation, respectively, in the present study. Reddy and Sivaiah (2001) reported similar IVDMD of 28.03 and 29.26 per cent for semi dwarf and medium varieties of paddy straw, respectively after 48 hours of incubation as per Tilley and Terry (1963) method. However, Liu and Orskov (2000) observed that cellulase (16 units per gram DM for 3 weeks) treatment of steam pretreated rice straw significantly increased the 24 hour IVOMD than that of untreated rice straw. As observed in the present study, Senthilkumar *et al.* (2007) also reported similar *in vitro* apparent DM digestibilty of paddy straw of 32.45 per cent.

5.2.1.2 Crude Protein

The *in vitro* CP disappearance of control feed, EFFBW, EFDBW, FBW, DBW, PS and BWIPS at 2 hours of incubation was 15.16, 14.70, 14.16, 13.18, 12.14, 10.44 and 11.17 per cent, respectively and at 72 hours of incubation was 56.06, 54.49, 52.59, 45.46, 41.01, 30.75 and 38.36 per cent, respectively in RUSITEC (Table 17 and Fig. 19).

The control feed, EFFBW and EFDBW had relatively higher CP disappearance in all incubation periods compared to FBW, DBW, PS and BWIPS in RUSITEC.

5.2.1.3 Crude Fibre

The *in vitro* CF disappearance of experimental feeds such as control feed, EFFBW, EFDBW, FBW, DBW, PS and BWIPS at 2 hours of incubation in RUSITEC was 22.00, 21.45, 20.35, 16.88, 15.81, 15.03 and 15.83 per cent, respectively (Table 18 and Fig. 20).

The CF disappearance at 24 hours incubation period was 46.72, 45.70, 44.22, 40.67, 40.32, 27.41 and 32.36 per cent for control feed, EFFBW, EFDBW, FBW, DBW, PS and BWIPS, respectively. During 72 hours of incubation, 62.54, 61.67, 60.17, 51.26, 44.87, 33.03 and 41.76 per cent crude fibre disappeared from control feed, EFFBW, EFDBW, FBW, DBW, PS and BWIPS, respectively.

5.2. 1. 4 Neutral Detergent Fibre

The *in vitro* NDF disappearance of control feed, EFFBW, EFDBW, FBW, DBW, PS and BWIPS at 2 hours of incubation in RUSITEC was 22.27, 18.28, 17.19, 14.96, 13.25, 12.57 and 14.16 per cent, respectively (Table 19 and Fig. 21).

The *in vitro* NDF disappearance values of DBW obtained in the present experiment (32.19 per cent) is in agreement with Batajoo and Shaver (1998) who reported the *in situ* NDF disappearance of 33.3 per cent in brewer's dried grain at 24 hours of incubation. But, the 24 hour *in vitro* NDF disappearance of distiller's dried grain was higher (43.6 per cent) than that obtained in the present study.

During 72 hours of incubation 54.89, 54.33, 52.99, 46.45, 44.16, 36.66 and 41.09 per cent NDF disappeared from control feed, EFFBW, EFDBW, FBW, DBW, PS and BWIPS, respectively. The FBW showed slightly higher NDF disappearance than DBW at all incubation periods.

Varga and Hoover (1983) reported that the *in situ* NDF disappearance of brewer's grain and distiller's grain were 50.8 and 76.6 per cent, respectively, values being higher than that obtained in the present study. They further reported that the *in situ* rate of degradation of brewer's grain and distiller's grain was 0.071 and 0.072 per hour, respectively. Firkins *et al.* (1985) reported that the *in situ* NDF disappearance rates were 4.4 and 3.7 per cent per hour for wet and dry corn distiller's grain, respectively in rumen cannulated steers.

Al-suwaeigh *et al.* (2002) reported *in situ* NDF disappearance of wet sorghum distiller's grain as 45.2 per cent, which is comparable to the findings of the present study while that of wet corn distiller's grain as 39 per cent which is lower than the present values obtained for fresh and dried brewery waste.

Ojowi *et al.* (1997) reported that *in situ* effective degradability of NDF was 46.3 per cent for wet distiller's grain and 38.3 per cent for wet brewer's grain. Mustafa *et al.* (2000b) reported that *in situ* effective degradability of NDF was highest for rye distiller's grain (47.0 per cent) followed by wheat (45.0 per cent) and triticale distiller's grain (43.9 per cent) and lowest for barley distiller's grain (34.2 per cent) in non-lactating Holstein cows. Mustafa *et al.* (2000a) also reported that *in situ* effective degradability of NDF was lower for barley distiller's grain (36.0 per cent) relative to wheat distiller's grain (45.4 per cent) in cows.

The *in vitro* NDF disappearance values of paddy straw obtained in the present RUSITEC experiment (36.66 per cent) is in agreement with Senthilkumar *et al.* (2007) who reported the *in vitro* NDF disappearance of 36.43 per cent.

5.2. 1. 5 Acid Detergent Fibre

The *in vitro* ADF disappearance of control feed, EFFBW, EFDBW, FBW, DBW, PS and BWIPS at 2 hours of incubation was 20.34, 15.46, 14.81, 13.95, 12.67, 11.45 and 13.24 per cent, respectively and at 72 hours of incubation was 53.02, 51.87, 51.09, 45.52, 43.76, 35.28 and 39.69 per cent, respectively in RUSITEC (Table 20 and Fig. 22). Senthilkumar *et al.* (2007) reported similar *in vitro* ADF disappearance of 35.61 per cent for paddy straw.

Ojowi *et al.* (1997) reported *in situ* effective degradability of ADF for wet brewer's grain and wet distiller's grain distiller's grain as 27.5 and 31.9 per cent, respectively.

5.2.1.6 Hemicellulose

The *in vitro* hemicellulose disappearance of control feed, EFFBW, EFDBW, FBW, DBW, PS and BWIPS at 2 hours of incubation was 20.54, 15.97, 14.88, 14.27, 12.83, 11.90 and 13.90 per cent, respectively and at 72 hours of incubation was 54.84, 53.89, 52.10, 46.62, 44.31, 37.11 and 39.35 per cent, respectively in RUSITEC (Table 21 and Fig. 23).

Senthilkumar *et al.* (2007) reported a higher *in vitro* hemicellulose disappearance for paddy straw (42.27 per cent) than those observed in the present RUSITEC study.

5.2.1.7 Cellulose

The *in vitro* cellulose disappearance of control feed, EFFBW, EFDBW, FBW, DBW, PS and BWIPS was 18.73, 14.42, 13.96, 13.63, 11.93, 8.43 and 12.10 per cent, respectively at 2 hours of incubation and was 52.50, 51.61, 50.99, 46.24, 42.19, 30.36 and 37.84 per cent, respectively at 72 hours of incubation in the RUSITEC (Table 22 and Fig. 24).

Durand *et al.* (1988) observed a significant improvement in the *in vitro* (RUSITEC) cellulose degradability when wheat straw was treated with sodium hydroxide. Addition of fibrolytic enzymes (30000 nova cellulase and 10000 nova xylanase units per kg DM) significantly improved the *in vitro* cellulose and hemicellulose disappearance of orchard grass hay (Dong *et al.*, 1999). A higher *in vitro* cellulose disappearance (39.51 per cent) of paddy straw than that is obtained in the present study (30.36 per cent) was reported by Senthilkumar *et al.* (2007).

From the present study, it can be seen that among the experimental feeds, control feed, EFFBW and EFDBW showed relatively higher *in vitro* disappearance of DM, CP, NDF, ADF, hemicellulose and cellulose in RUSITEC. The paddy straw showed lowest *in vitro* disappearance of DM, CP, CF, NDF, ADF, hemicellulose and cellulose. However, the BWIPS showed slightly higher *in vitro* disappearance of all the components than the paddy straw. The FBW and DBW showed intermediary *in vitro* disappearance of DM, CP, CF, NDF, ADF, hemicellulose and cellulose and the disappearance of all the components at all incubation periods were higher for FBW than the DBW.

5.2.2 In Vitro Degradability Studies in RUSITEC

5.2.2.1 Dry Matter

The data on DM fractions of soluble '*a*', degradable '*b*', rate of degradation '*c*' and the percentage *in vitro* effective degradability of DM of control feed, EFFBW, EFDBW, FBW, DBW, PS and BWIPS in RUSITEC are presented in Table 23 and illustrated in Fig. 25.

5.2.2.1.1 Soluble 'a' Fraction

From the present study, it could be seen that the soluble '*a*' fraction of control feed, EFFBW and EFDBW (23.10, 21.79 and 20.05 per cent, respectively) were similar, but were higher than that of FBW, DBW, PS and BWIPS (13.68, 10.98, 10.33 and 11.85 per cent, respectively).

Armentano *et al.* (1986) also reported similar *in vitro* DM '*a*' fraction for wet brewer's grain (11.0 per cent) but, lower value of 4.0 per cent for dried brewer's grain than those obtained in the present RUSITEC experiment. Orskov *et al.* (1992) also reported that the '*a*' fraction of DM of brewer's grain was 15.2 per cent. However, Batajoo and Shaver (1998) reported higher '*a*' fraction for distiller's dried grain (40.2 per cent) and lower value for brewer's dried grain (14.5 per cent).

On contrary, Armentano *et al.* (1986) reported a higher *in situ* DM '*a*' fraction for wet brewer's grain (29.0 per cent) and dried brewer's grain (20.0 per cent) than those observed for brewery waste in the present RUSITEC experiment. Ojowi *et al.* (1997) also reported higher DM '*a*' fraction for wet brewer's grain (23.8 per cent) and wet distiller's grain (25.1 per cent) than those observed in the present study. Mustafa *et al.* (2000a) also reported higher DM '*a*' fraction for barley based distiller's grain (21.6 per cent) and wheat based distiller's grain (25.1 per cent) than those values obtained for brewery waste in the present study.

Aman and Hesselman (1984) reported that soluble '*a*' fraction contained soluble sugars such as glucose, fructose, sucrose, and fructans, as well as soluble non starch polysaccharides such as arabinose, xylose, mannose, galactose and uronic acids. Broderick, (1987) and Broderick and Craig (1989) reported that the small feed particles could be physically expelled from the bag during soaking prior to incubation and washing which would overestimate the amount leaving the bag due to solubilization. Also, soluble protein or particles exiting the bag do not necessarily reflect degradation. They also reported that bovine serum albumin, which is soluble in water, is not highly degradable when incubated in rumen liquor.

Compared to other experimental feeds, the paddy straw had the lowest DM '*a*' fraction. The BWIPS showed slightly higher '*a*' value than paddy straw alone. Reddy and Sivaiah (2001) reported that there was a significant difference in '*a*' fraction between fifteen varieties of paddy straw (semi dwarf and medium) and it ranged from 8.33 to 14.06 per cent, which agrees well with that obtained in the present study. Sohane and Singh (2001) observed the rapidly soluble fraction of paddy straw as 14.1 per cent.

5.2.2.1.2 Degradable 'b' Fraction

The higher '*b*' values of 40.68, 40.57, 40.65, 39.00 and 35.78 per cent for control feed, EFFBW, EFDBW, FBW and DBW, respectively, obtained in the present study indicated that these experimental feeds are high in potentially degradable DM and are low in undegradable dietary DM contents compared to paddy straw and BWIPS, which had lower '*b*' values of 22.91 and 30.40 per cent, respectively.

Among the experimental feeds, paddy straw showed lowest dry matter '*b*' fraction. The BWIPS showed the '*b*' fraction value greater than that of paddy straw. The FBW showed, slightly higher DM '*b*' fraction compared to DBW.

However, Armentano *et al.* (1986) reported higher *in situ* and *in vitro* DM '*b*' fraction for wet brewer's grain (41.0 and 47.0 per cent, respectively) and dried brewer's grain (42.0 and 38.0 per cent, respectively) than those obtained for brewery waste in the present RUSITEC experiment. Orskov *et al.* (1992) reported that ruminal *in situ* effective degradability of DM '*b*' fraction of brewer's grain was 50.7 per cent. Similarly, Ojowi *et al.* (1997) reported higher DM '*b*' fraction for wet brewer's grain (48.6 per cent) and wet distiller's grain (60.5 per cent) than those obtained for brewery waste in the present study. Batajoo and Shaver (1998) reported that DM '*b*' fraction was 57.2 per cent for brewer's dried grain and 44 per cent for distiller's dried grain. Similarly, Mustafa *et al.* (2000a) reported that higher DM '*b*' fraction for barley based distiller's grain (49.8 per cent) and wheat distiller's grain (60.7 per cent) than those obtained for brewery waste in the present study.

Reddy and Sivaiah (2001) reported that there was a significant difference in '*b*' fraction between fifteen varieties of paddy straw (semi dwarf and medium) which ranged from 51.75 to 56.97 per cent, which was higher than those obtained for paddy straw in the present study. Sohane and Singh (2001) also reported that the degradable fraction '*b*' of paddy straw was 35.05 per cent which were higher than the '*b*' value observed for paddy straw in the present study.

Trach (2003b) reported similar DM '*b*' fraction of 47.1 and 47 per cent in untreated and treated rice straw (3 per cent lime + 2 per cent urea) with 10 per cent wet brewer's grain using rumen fistulated animals.

5.2.2.1.3 Rate of Degradation 'c'

The rate of degradation '*c*' of DM was higher in control feed, EFFBW and EFDBW (0.51, 0.51 and 0.50 per cent / hour, respectively) in RUSITEC. Among the feeds experimented in RUSITEC, the paddy straw showed minimum rate of degradation '*c*' value (0.21 per cent / hour). The BWIPS showed slightly higher rate of degradation (0.33 per cent / hour) when compared to paddy straw.

The FBW and DBW showed intermediary rate of degradation among the feeds experimented and FBW showed higher rate of degradation (0.45 per cent / hour) than the DBW (0.37 per cent / hour). Similarly, Armentano *et al.* (1986) reported that ruminal *in situ* effective degradability of DM '*c*' fraction for wet brewer's grain was 0.048/hour and dried brewer's grain was 0.039/hour. However, they further reported that ruminal *in vitro* effective degradability of DM '*c*' fraction for wet brewer's grain was 0.042/hour and dried brewer's grain was 0.058/hour.

Orskov *et al.* (1992) reported that ruminal *in situ* effective degradability of DM '*c*' fraction of brewer's grain was 0.0282/hour. Batajoo and Shaver (1998) also reported similar DM degradation rate (*c*) of 0.049/hour for brewer's dried grain and distiller's dried grain.

Ojowi *et al.* (1997) reported that higher rate of degradation for DM for wet brewer's grain (5.3 per cent / hour) and wet distiller's grain (4.1 per cent / hour) than those values obtained for brewery waste in the present RUSITEC experiment. Similarly, Mustafa *et al.* (2000a) reported higher rate of degradation for DM for barley based distiller's grain (4.1 per cent / hour) and wheat based distiller's grain (3.5 per cent / hour) and Mustafa *et al.* (2000b) reported the rate of degradation for DM (3.7 per cent / hour) in distiller's grain which were higher than the values obtained for brewery waste in the present study.

Colombatto (2000) stated that treatment of forage with fibrolytic enzyme mixtures increased the rate of degradation *in vitro*. Reddy and Sivaiah (2001) reported that there was a significant difference of '*c*' fraction between fifteen varieties of paddy straw (semi dwarf and medium) which ranged from 0.0352 to 0.0441 per hour. Sohane and Singh (2001) also reported similar degradation rate (*c*) of 0.03 per hour for paddy straw. Trach (2003b) reported DM '*c*' fraction of untreated and treated rice straw (3 per cent lime + 2 per cent urea) when supplemented with 10 per cent wet brewer's grain were (0.040 and 0.042/hour respectively) significantly higher compared to those with non-supplemented (0.035 and 0.030/hour, respectively). Vinil and Balakrishnan (2008) reported that rate of degradation for DM was 0.035 to 0.04 per hour for different varieties of paddy straw.

5.2.2.1.4 Effective Degradability

The control feed, EFFBW and EFDBW had higher *in vitro* effective DM degradability (61.74, 60.32 and 58.62 per cent, respectively) than other experimental feeds such as FBW, DBW, PS and BWIPS (50.48, 44.34, 30.59 and

39.93 per cent, respectively) in RUSITEC. Paddy straw showed lowest effective degradability of DM (30.59 per cent) compared to other feeds experimented in RUSITEC. The BWIPS showed higher effective DM degradability (39.93 per cent) compared to paddy straw alone (30.59 per cent). The FBW showed higher DM degradability (50.48 per cent) than DBW (44.34 per cent).

Ojowi *et al.* (1997) reported that *in situ* effective degradability of DM for wet brewer's grains and wet distiller's grains was 48.7 and 52.4 per cent, respectively. Mustafa *et al.* (2000a) also reported similar in *situ* effective ruminal degradability of DM in cows for wheat distiller's grain (52.2 per cent) and barley distiller's grain (43.9 per cent) and were comparable with the results obtained in the present RUSITEC study. Mustafa *et al.* (2000b) reported that *in situ* effective ruminal degradability of DM in non-lactating Holstein cows was highest for rye distiller's grain (52.8 per cent) and lowest for barley distiller's grain (38.1 per cent). Also the ruminal degradability of DM was higher for triticale (47.4 per cent) than for wheat distiller's grain (43.1 per cent).

Trach (2003b) studied the 48 hour *in situ* DM degradability of rice straw based diet supplemented with 10 per cent wet brewer's grains in fistulated animals and found significant improvement from 48.3 to 55.4 per cent. He further reported that supplementation of 10 per cent wet brewer's grains to treated (3 per cent lime plus 2 per cent urea) rice straw significantly increased the 48 hour DM degradability from 50.9 to 56.4 per cent in fistulated animals.

On contrary, Reed *et al.* (2006) reported that the IVOMD of corn distiller's dried grains was significantly reduced (79.33 per cent) compared to control diet containing soyabean meal and wheat middlings (85.67 per cent) as per Tilley and Terry (1963) method.

From the present study, paddy straw had the *in vitro* effective DM degradability of 30.59 per cent in RUSITEC, whereas Sohane and Singh (2000) reported the *in sacco* DM degradability of different varieties of paddy straw at 72 hours of incubation ranged from 42.76 to 59.67 per cent. Liu and Orskov (2000) also observed that cellulase (16 units per gram DM for 3 weeks) treatment of steam pretreated rice straw significantly increased the 24 hour IVOMD than that of untreated rice straw. Reddy and Sivaiah (2001) reported higher *in sacco* DM degradability for semi dwarf and medium varieties of paddy straw as 60.11 and 60.27 after 72 hours of incubation, respectively than those observed in the present study. They further reported that the IVDMD of semi dwarf and medium varieties of paddy straw was 28.03 and 29.26 per cent, respectively after 24 hours of incubation as per Tilley and Terry (1963) method. Similarly, Fazali *et al*. (2006) reported that *in vitro* DM degradability of wheat straw was 28.1 per cent by Tilley and Terry (1963) method. However, Vinil and Balakrishnan (2008) reported lower *in vitro* effective DM degradability of 24.39 per cent for paddy straw in RUSITEC than those obtained in the present RUSITEC experiment (30.59 per cent).

5.2.2.2 Crude Protein

The data on CP fractions, soluble '*a*', degradable '*b*' and rate of degradation '*c*' and the percentage *in vitro* effective degradability of CP of control feed, EFFBW, EFDBW, FBW, DBW, PS and BWIPS in RUSITEC are presented in Table 24 and illustrated in Fig. 26.

5.2.2.2.1 Soluble 'a' Fraction

From the present study, it can be seen that control feed had the highest '*a*' value (10.87 per cent), followed by EFFBW, EFDBW and FBW (9.31, 9.28 and 8.80 per cent, respectively) indicating its higher level of rapidly soluble CP percentage as compared to DBW, PS and BWIPS (7.70, 6.73 and 7.95 per cent, respectively). Lowest '*a*' value was observed for paddy straw when compared to other experimental feeds. The BWIPS showed slightly higher '*a*' value than paddy straw.

Armentano *et al.* (1986) reported higher *in situ* and *in vitro* CP '*a*' fraction for wet brewer's grain (51.0 and 27.0 per cent, respectively) and dried brewer's grain (19.0 and 23.0 per cent, respectively) than those obtained for brewery waste in the present RUSITEC experiment. Ojowi *et al.* (1997) also reported higher CP '*a*' fraction for wet brewer's grain (26.5 per cent) and wet distiller's grain (37.1 per cent) than those observed in the present study. Mustafa *et al.* (2000a) also reported higher CP '*a*' fraction for barley based distiller's grain (19.8 per cent) and wheat based distiller's grain (34.1 per cent) than those values obtained for brewery waste in the present study.

Batajoo and Shaver (1998) reported that CP '*a*' fraction was similar for brewer's dried grain and distiller's dried grain (17 per cent), but the values were higher than the values obtained for brewery waste in the present RUSITEC experiment.

5.2.2.2.2 Degradable 'b' Fraction

The higher '*b*' values of 45.19, 45.18 and 43.31 per cent for control feed, EFFBW, EFDBW respectively, obtained in the present study indicate that these three experimental feeds are high in potentially degradable CP and are low in undegradable dietary CP contents compared to FBW, DBW, paddy straw and BWIPS, which had lower '*b*' values of 36.66, 33.30, 24.02 and 30.41 per cent, respectively.

Among the experimental feeds, paddy straw showed lowest '*b*' value for crude protein. The BWIPS showed the CP '*b*' value greater than paddy straw alone. The FBW showed slightly higher CP '*b*' value than DBW.

Armentano *et al.* (1986) reported higher *in situ* and *in vitro* CP '*b*' fraction for wet brewer's grain (39.0 and 50.0 per cent, respectively) and dried brewer's grain (51.0 and 30.0 per cent, respectively) than those obtained for brewery waste in the present RUSITEC experiment. Ojowi *et al.* (1997) also reported higher CP '*b*' fraction for wet brewer's grain (46.0 per cent) and wet distiller's grain (50.2 per cent) than those obtained for brewery waste in the present study. Similarly, Mustafa *et al.* (2000a) reported that CP '*b*' fraction for barley based distiller's grain (68.4 per cent) and wheat based distiller's grain (59.4 per cent) were higher than those obtained for brewery waste in the present study (36.66 per cent).

Batajoo and Shaver (1998) also reported higher CP '*b*' fraction for brewer's dried grain (64.3 per cent) and distiller's dried grain (56.1 per cent) than values obtained for brewery waste in the present RUSITEC experiment.

5.2.2.2.3 Rate of Degradation 'c'

The rate of degradation '*c*' of CP was higher in control feed, EFFBW and EFDBW (0.56, 0.55 and 0.54 per cent / hour, respectively) in RUSITEC. Among the feeds experimented in RUSITEC, the paddy straw showed minimum rate of degradation '*c*' value (0.28 per cent / hour) for CP. The BWIPS showed slightly higher rate of degradation of CP (0.38 per cent / hour) when compared to paddy straw. The FBW and DBW showed, intermediary rate of degradation (0.42 and 0.38 per cent / hour, respectively) among the feeds experimented and FBW showed higher rate of degradation of CP than the DBW.

Armentano *et al.* (1986) reported that *in situ* and *in vitro* rate of degradation of CP '*c*' fraction for wet brewer's grain were 0.066 and 0.055/hour, respectively and that of dried brewer's grain were 0.042 and 0.052/hour, respectively. Similar CP degradation rate of 0.049/hour for distiller's dried grain (Grings *et al.* (1992), but slightly faster CP degradation rate of 0.068/h was reported by Nocek (1987) and Batajoo and Shaver (1998) for brewer's dried grain.

Ojowi *et al.* (1997) also reported higher rate of degradation for CP for wet brewer's grain (5.9 per cent / hour) and wet distiller's grain (8.8 per cent / hour) than observed in the present experiment. Similarly, Mustafa *et al.* (2000a) reported higher rate of degradation of CP for barley based distiller's grain (7.8 per cent / hour) and wheat distiller's grain (6.3 per cent / hour) than those obtained for brewery waste in the present RUSITEC experiment.

5.2.2.2.4 Effective Degradability

The control feed, EFFBW and EFDBW had higher *in vitro* effective CP degradability (53.97, 52.37 and 50.51 per cent, respectively) than other experimental feeds such as FBW, DBW, PS and BWIPS (43.25, 38.79, 28.64 and 36.32 per cent, respectively) in RUSITEC. In the present study, paddy straw showed lowest effective degradability of CP (28.64 per cent) compared to other feeds experimented in RUSITEC. The BWIPS showed higher effective CP degradability (36.32 per cent) than the paddy straw (28.64 per cent). The FBW showed higher CP degradability (43.25 per cent) than DBW (38.79 per cent).

Carroll *et al.* (1997) reported lower *in situ* undegraded intake protein value of barley distiller's grain and corn distiller's grain in lactating Holstein cows, which ranged from 37 to 44 and 42 to 44 per cent of CP, respectively than that of FBW and DBW obtained (56.75 and 61.21 per cent, respectively) in the present study.

Ojowi *et al.* (1997) reported higher *in situ* effective degradability of CP for wet brewer's grain (54.9 per cent) and wet distiller's grain (69.1 per cent). Similarly, higher *in situ* effective ruminal degradability of CP in cows for wheat, barley and rye distiller's grain was reported by Mustafa *et al.* (2000a) and Mustafa *et al.* (2000b) than the values for brewery waste obtained in the present study. Peter *et al.* (2000) reported that CP degradation of corn distiller's grain in ruminally cannulated beef steers was 57.6 per cent at 48 hours. Geetha (2007) reported a lower effective CP degradability (24.76 per cent) of wet distiller's grain than those obtained for brewery waste in the present RUSITEC experiment (43.25 per cent).

The over all results of *in vitro* degradability studies using RUSITEC indicates that control feed, EFFBW and EFDBW had higher '*a*' and '*b*' fractions, rate of degradation '*c*' and *in vitro* effective degradability of DM and CP than other experimental feeds such as fresh brewery waste (FBW), dried brewery waste (DBW), paddy straw and brewery waste incorporated paddy straw (BWIPS).

Paddy straw showed lowest *in vitro* '*a*' and '*b*' fractions, rate of degradation '*c*' and effective degradability of DM and CP compared to other feeds experimented. The BWIPS showed higher *in vitro* effective DM and CP degradability compared to paddy straw. The FBW showed higher DM and CP '*a*' and '*b*' fractions, rate of degradation '*c*' and effective degradability than DBW. The FBW and DBW showed intermediary in values for *a*, *b, c* and *in vitro* effective degradability of DM and CP compared to other experimental feeds.

A low *in vitro* DM and CP disappearance of 33.24 and 30.75 per cent, respectively, was recorded at 72 hours of incubation (effective degradability of 30.59 and 28.64 per cent, respectively for DM and CP) of paddy straw, whereas the BWIPS had higher *in vitro* DM and CP disappearance of 42.25 and 38.36 per cent, respectively at 72 hours of incubation (with effective degradability of 39.93 and 36.32 per cent, respectively for DM and CP) in RUSITEC.

5.2.3 *In Vitro* Rumen Fermentation Characteristics

5.2.3.1 In Vitro Total Gas Production

The mean *in vitro* total gas production (ml) of experimental feeds such as control feed, EFFBW, EFDBW, FBW, DBW, PS and BWIPS at 2, 6, 12, 24, 48 and 72 hours of incubation in RUSITEC are presented in Table 25 and graphically illustrated in Fig. 27.

The *in vitro* total gas production of control feed, EFFBW, EFDBW, FBW, DBW, PS and BWIPS at 2 hours of incubation was 330, 300, 350, 395, 133, 155 and 156 ml, respectively and at 72 hours of incubation was 2486, 3925, 3860, 2415, 3585, 2435 and 3115 ml in RUSITEC (Table 25 and Fig. 27).

During initial incubation periods (2, 6, 12 and 24 hours) the control feed, EFFBW, EFDBW and FBW showed higher *in vitro* gas production than other experimental feeds such as DBW, paddy straw and BWIPS. This may be due to the presence of easily degradable carbohydrates, degradation of which produced more gas during initial hours. During later incubation periods (48 and 72 hours) DBW, paddy straw and BWIPS showed higher *in vitro* gas production than other experimental feeds such as control feed, EFFBW, EFDBW and FBW.

Upto 24 hours of incubation, the FBW showed higher gas production than DBW, after wards it showed a lower total gas production. Srinivas and Singh (1998) reported that the total gas production was directly proportional to the degradability of the nutrients.

From the present study, the total gas production on fermentation of FBW was lower as compared to DBW, which is due to higher NFE present in the FBW (Gandi *et al.* 1995). Geetha (2007) reported that the total gas production was higher in preserved distiller's grain using propionic acid (1.05 litre/day) than that of fresh distiller's grain (0.65 litre/day) in RUSITEC.

Durand *et al.* (1988) observed a significant increase in the *in vitro* total gas production using RUSITEC when wheat straw was treated with ammonia (0.87 l/day) and sodium hydroxide (1.31 l/day) compared to those with untreated (0.86 l/day) wheat straw. Similarly, Liu and Orskov (2000) found that the cellulase (16 units) treatment of steam pre-treated paddy straw significantly improved the 24 hours *in vitro* cumulative gas production (27.50 ml) and rate of gas production (4.45 per cent per hour).

5.2.3.2 Carbon dioxide (CO_2) Production

The mean carbon dioxide (CO_2) production of experimental feeds such as control feed, EFFBW, EFDBW, FBW, DBW, PS and BWIPS at different periods of incubation (2, 6, 12, 24, 48 and 72 hours) in RUSITEC is presented in Table 26 and graphically illustrated in Fig. 28.

The *in vitro* CO_2 production of control feed, EFFBW, EFDBW, FBW, DBW, PS and BWIPS at 2 hours of incubation was 57.50, 57.50, 55.00, 55.00, 55.00, 47.50 and 48.75 per cent, respectively and at 72 hours of incubation was 57.50, 60.00, 57.50, 52.50, 57.50, 57.50 and 50.00 per cent, respectively. From the present study, not much difference was observed in CO_2 production (47.5 to 60.0 per cent) at different periods of incubation in the experimental feeds incubated in RUSITEC.

Gizzi and Givens (2001) and Holmberg (2004) reported that the addition of both wet and dry distiller's grain in total mixed ration significantly reduced the CH_4 emission by 20 to 30 per cent in cows. Similarly, Smith (2005) observed that feeding of fermented grains (brewer's grain and distiller's grain) to ruminants, significantly reduced the CH_4 emission by 20 to 50 per cent in emission factor (Methane/gross energy intake, percentage). Though earlier reports (Wood and Knipmeyer, 1998; Gizzi and Givens, 2001 and Holmberg, 2004) had stated that CH_4 losses from distiller's grain are low, no such evidence has been obtained from this study.

The *in vitro* CO_2 : CH_4 ratio obtained in the present study (0.91 to 1.50) was lower than those reported by Senthilkumar *et al.* (2007) for paddy straw (1.65) indicating higher CO_2 produced from paddy straw than methane production.

5.2.3.3 In Vitro Ruminal pH

The mean *in vitro* ruminal pH of experimental feeds such as control feed, EFFBW, EFDBW, FBW, DBW, PS and BWIPS at different incubation periods (2, 6, 12, 24, 48 and 72 hours) in RUSITEC is presented in Table 27 and graphically illustrated in Fig. 29.

The *in vitro* ruminal pH of control feed, EFFBW, EFDBW, FBW, DBW, PS and BWIPS at 2 hours of incubation were 7.04, 7.21, 7.23, 7.31, 7.22, 7.00 and 7.24, respectively and at 72 hours of incubation were 7.20, 7.00, 7.19, 7.08, 6.98, 7.02 and 6.94, respectively.

In the present study, not much difference was observed in pH at different periods of incubation of the experimental feeds, and the values observed were from 6.89 to 7.45. However, Geetha (2007) reported that the *in vitro* ruminal pH (RUSITEC) for complete feed containing 10 to 40 per cent distiller's grain was 6.86 to 6.91.

Trach (2003b) also studied the rumen liquor pH of rice straw based diet supplemented with 10 per cent wet brewer's grains in fistulated animals and found significant reduction due to the addition of wet brewer's grain from 7.09 to 6.53. He further reported that supplementation of 10 per cent wet brewer's grains to treated rice straw (3 per cent lime + 2 per cent urea) significantly reduced the pH from 6.71 to 6.34 in fistulated animals.

Wang *et al.* (2001) found significant difference in pH when barley straw was mixed with four nitrogen supplements such as fish meal, casein, soyabean meal and urea (6.58, 6.56, 6.56 and 6.83, respectively) in RUSITEC.

5.2.3.4 In Vitro Ruminal Ammonia Nitrogen (NH_3-N) Production

The mean *in vitro* ruminal NH_3-N (mg/100 ml) of experimental feeds such as control feed, EFFBW, EFDBW, FBW, DBW, PS and BWIPS at different incubation periods (2, 6, 12, 24, 48 and 72 hours) in RUSITEC are presented in Table 28 and graphically illustrated in Fig. 30.

The *in vitro* NH_3-N concentration of control feed, EFFBW, EFDBW, FBW, DBW, PS and BWIPS at 2 hours of incubation was 17.12, 19.79, 12.64, 34.92, 11.48, 0.66 and 4.33 mg/100 ml, respectively and at 72 hours of incubation was 20.28, 17.20, 9.48, 32.64, 11.27, 0.98 and 2.22 mg/100 ml, respectively.

Both the brewery waste containing experimental feeds such as EFFBW and EFDBW had lower NH_3-N concentration (16.14 and 11.38 mg/100 ml, respectively) compared to that of control feed (25.64 mg/100 ml) at 24 hours of incubation. The reason being the rumen degradable protein (RDP) of control feed was relatively higher than the experimental feed containing brewery waste such as EFFBW and EFDBW.

In the present study, the FBW showed highest *in vitro* NH_3-N production throughout the incubation periods of 72 hours, followed by control feed and EFFBW. The paddy straw had lowest NH_3-N production at different incubation periods compared to other experimental feeds. However, the BWIPS showed slightly higher NH_3-N production at all incubation periods than paddy straw. The EFDBW and DBW showed intermediary in NH_3-N production at all periods of incubation compared to other experimental feeds in RUSITEC.

Trach (2003b) reported that there was no significant difference in ruminal NH_3-N concentration of untreated and treated rice straw (3 per cent lime + 2 per cent urea) based diet supplemented with 10 per cent wet brewer’s grains, values being 26.6 and 24.4 mg/100 ml, respectively, in fistulated animals.

5.2.3.5 In Vitro Ruminal Total Volatile Fatty Acids (TVFA)

The mean *in vitro* total volatile fatty acids (TVFA) concentration (mmol/l) of experimental feeds such as control feed, EFFBW, EFDBW, FBW, DBW, PS and BWIPS at different incubation periods (2, 6, 12, 24, 48 and 72 hours) in RUSITEC are presented in Table 29 and graphically illustrated in Fig. 31.

The *in vitro* TVFA concentration of control feed, EFFBW, EFDBW, FBW, DBW, PS and BWIPS was 74.25, 40.75, 39.00, 27.00, 29.75, 27.50 and 27.00 mmol/l, respectively at 2 hours of incubation and was 64.00, 44.75, 59.50, 41.75, 29.00, 29.75 and 27.13 mmol/l, respectively at 72 hours of incubation.

In the present study, the TVFA concentration was comparatively higher in control feed, EFFBW and EFDBW at different periods of incubation than FBW, DBW, PS and BWIPS. The FBW had higher TVFA concentration than DBW in later incubation hours (12, 24, 48 and 72 hours). From the present study, the TVFA concentration was comparatively lower in paddy straw at different period of incubation than other experimental feeds in RUSITEC.

The EFFBW had higher TVFA concentration during initial incubation hours (2, 6 and 12 hours) than EFDBW. The EFDBW had higher TVFA concentration during later incubation hours (24, 48 and 72 hours). The EFFBW had lower TVFA concentration than control feed at 2, 6, 48 and 72 hours, whereas at 12 and 24 hours, the values were comparable. During later incubation hours (12, 24, 48 and 72 hours) the FBW had higher TVFA concentration (33.50, 51.25, 43.25 and 41.75 mmol/l, respectively) than DBW (29.75, 34.75, 34.00 and 29.00 mmol/l, respectively) in RUSITEC.

Trach (2003b) also found significant improvement in TVFA concentration from 68 to 94 mmol/l due to addition of 10 per cent wet brewer's grains to rice straw based diet in fistulated animals. He further reported that supplementation of 10 per cent wet brewer's grain to treated rice straw (3 per cent lime plus 2 per cent urea) significantly increased the TVFA concentration from 93 to 114 mmol/l in fistulated animals.

5.2.3.6 In Vitro Ruminal Volatile Fatty Acids (VFA) Fractions

The mean *in vitro* volatile fatty acids (VFA) fractions such as acetate, propionate, iso butyrate, butyrate and valerate concentration (mmol/l) of experimental feeds such as control feed, EFFBW, EFDBW, FBW, DBW, PS and BWIPS at different incubation periods (2, 6, 12, 24, 48 and 72 hours) in RUSITEC are presented in Table 30 and graphically illustrated in Fig. 32, 33, 34, 35 and 36, respectively.

During initial incubation periods (2, 6, 12 and 24 hours) all the experimental feeds showed higher concentration of acetate than in later incubation periods (48 and 72 hours). At 24 hours of incubation, the EFDBW showed highest acetate concentration (349.64 mmol/l) compared to other experimental feeds at different incubation periods. The paddy straw and BWIPS showed lower acetate and propionate concentration at different incubation periods. Senthilkumar *et al.* (2007) also reported lower *in vitro* acetate and propionate concentration of 36.27 and 6.97 mmol/l, respectively, for paddy straw than those observed in the present RUSITEC experiment at 12 hours of incubation (64.49 and 30.54 97 mmol/l, respectively).

In the present study, butyrate and valerate were absent in paddy straw at all incubation periods. But, Senthilkumar *et al.* (2007) reported that *in vitro* butyrate concentration for paddy straw at 48 hours of incubation was 4.11 mmol/l. Similarly, BWIPS also had no butyrate and valerate concentration at all incubation periods (except butyrate concentration of 15.50 mmol/l at 2hours and 0.30 mmol/l at 72 hours) in RUSITEC.

The control feed, EFFBW, EFDBW, FBW and DBW had higher propionate concentration than paddy straw and BWIPS at all incubation periods. Similar to acetate, the EFDBW showed highest propionate concentration (266.54 mmol/l) at 24 hours of incubation compared to other experimental feeds at all incubation periods.

The control feed, EFFBW, EFDBW, FBW and BWIPS had higher iso butyrate concentration compared to DBW and paddy straw at all incubation periods (2, 6, 12, 24, 48 and 72 hours). The BWIPS had more iso butyrate concentration than DBW at all incubation periods in RUSITEC.

Similar to acetate and propionate, the EFDBW contained highest iso butyrate concentration (90.63 mmol/l) at 24 hours of incubation in RUSITEC among the experimental feeds at all incubation periods. The butyrate and valerate were absent in paddy straw at different incubation periods, while BWIPS contained butyrate only at 2 hours of incubation (15.50 mmol/l) and valerate only at 12 hours of incubation (1.69 mmol/l). The other experimental feeds such as control feed, EFFBW, EFDBW, FBW and DBW contained butyrate and valerate with varying levels at different incubation hours.

Our values are in agreement with the values of Murdock *et al.* (1981) who reported that molar per cent of acetate, propionate and butyrate concentration were 60.9 vs 62.2, 23.6 vs 22.0 and 15.5 vs 15.8, respectively, in lactating dairy cows fed with diets containing wet brewer's grain as a concentrate replacement at 15 vs 30 per cent of the total ration DM.

They further found A:P ratio of 2.7 and 2.9 in diets containing wet brewer's grains as a concentrate replacement at 15 and 30 per cent of the total ration DM, which was approximately 25 and 50 per cent of the total concentrate DM in rations of lactating dairy cows, respectively. They found no effect on molar ratio of volatile fatty acids when cows were fed with rations containing wet brewer's grain. However, there was a non significant trend for higher A:P ratio for rations containing wet brewer's grain.

Palmquist and Conrad (1982) observed that A:P ratio was considerably increased when cows were fed with diets containing dried distiller's grains and solubles, reflecting a lower concentration of readily fermentable starch in the feedstuff. They further observed that the concentration of acetate, propionate and butyrate were 63.1, 23.6 and 13.5 molar per cent, respectively and A:P ratio was 2.68 in dairy cows fed with diets containing distiller's dried grains with solubles.

Nichols *et al.* (1998) also found significant increase in acetate and reduction in the propionate concentration in cows fed with diets containing distiller's grain compared to those fed with soybean meal based diet in agreement with Schingoethe *et al.* (1999). Peter *et al.* (2000) also observed that the acetate, propionate and butyrate concentrations were 32.6, 14.2 and 6.8 mmol/l, respectively in beef cattle heifer fed with diets containing corn dried distiller's grain, whereas, Al-suwaeigh *et al.* (2002) observed 53.9, 24.9, 16.38 mmol/l of acetate, propionate, butyrate, respectively in dairy cows fed with corn dried distiller's grain based diet. They further reported acetate, propionate and butyrate concentration of 54.4, 25.2, 15.5 mmol/l, respectively in dairy cows fed with diets containing sorghum dried distiller's grain.

In agreement with present experiment, Anderson *et al.* (2006) found significant improvement in the butyrate concentration when lactating dairy cows were fed with diets containing dried corn distiller's grain compared to those with wet corn distiller's grain. However, no significant difference was observed in acetate and propionate concentration and A:P ratio of total mixed ration as a result of incorporation of distiller's grain in the diet for lactating dairy cows compared to those fed with corn and soyabean meal based control diet (Al-Suwaiegh *et al*., 2002 and Anderson *et al*., 2006).

From the overall results on *in vitro* rumen fermentation characteristics using RUSITEC indicated that during initial incubation hours (2, 6 and 12 hours), the control feed, EFFBW, EFDBW and FBW showed higher *in vitro* gas production than other experimental feeds such as DBW, PS and BWIPS, whereas, during later incubation hours (48 and 72 hours), the DBW, PS and BWIPS showed higher *in vitro* gas production than other experimental feeds. Upto 24 hours of incubation, the FBW showed higher gas production than DBW, afterwards it showed lower total gas production. Not much difference was observed in *in vitro* carbon dioxide (CO_2) production and pH at all periods of incubation in all the seven experimental feeds incubated in RUSITEC.

The TVFA concentration was comparatively higher in control feed, EFFBW and EFDBW at all periods of incubation than FBW, DBW, PS and BWIPS. The FBW had higher TVFA concentration than DBW in later incubation hours (12, 24, 48 and 72 hours). The EFFBW had higher TVFA concentration during initial incubation hours (2, 6 and 12 hours) than DBW, while the DBW had higher TVFA concentration during later incubation hours (24, 48 and 72 hours).

The FBW showed highest *in vitro* NH_3-N production at all incubation periods, followed by control feed and EFFBW. The paddy straw had lowest NH_3-

N production at all incubation periods among the experimental feeds. However, the BWIPS showed slightly higher NH_3-N production at all incubation periods than paddy straw. The EFFBW and DBW showed intermediary in NH_3-N production at all periods of incubation compared to other experimental feeds in RUSITEC.

During initial incubation periods (2, 6, 12 and 24 hours) all the experimental feeds showed higher acetate concentration than later incubation hours (48 and 72 hours). The EFDBW showed, highest acetate, propionate and iso butyrate concentration (349.64, 266.54 and 90.63 mmol/l, respectively) at 24 hours of incubation, compared to other experimental feeds at all incubation periods. The paddy straw and BWIPS showed lower acetate and propionate concentration at all incubation periods. The control feed, EFFBW, EFDBW, FBW and BWIPS showed higher iso butyrate concentration compared to DBW and paddy straw at all incubation periods. The BWIPS had more iso butyrate concentration than DBW throughout the incubation periods of 72 hours in RUSITEC.

SUMMARY

6. SUMMARY

Two experiments were conducted, one to assess the effect of brewery waste on nutrient digestibility of paddy straw based diet in lactating cows and the other to study the *in vitro* disappearance, degradability and rumen fermentation characteristics of various experimental feeds in Rumen Simulation Technique (RUSITEC).

In animal study, eighteen early lactating cross bred cows within 20 days of lactation were selected from University Livestock Farm and Fodder Research and Development Scheme (ULF and FRDS) as the experimental animals. The cows were grouped into three as uniform as possible with regard to their age, body weight, parity, previous milk yield and stage of lactation. They were randomly allotted to the three dietary treatments T1, T2 and T3. The three dietary treatments were: T1, control concentrate mixture and paddy straw as roughage; T2 and T3, experimental concentrate mixture containing brewery waste at 25% level (on DM basis). In T2, the brewery waste was fed separately on wet basis, while in T3, the brewery waste to be incorporated in the concentrate mixture was treated with required quantity of paddy straw three days before feeding. An adaptation period of seven days was given to each animal. The cows were fed as per NRC (1989) standards.

The experiment was carried out for a period of 60 days and the animals were maintained under similar managemental conditions. Feeding was done twice daily and brewery waste was fed along with afternoon feeding. Individual records of daily intake of concentrate mixture, paddy straw and brewery waste and daily milk production of the experimental animals were maintained throughout the experiment. Milk samples collected at fortnightly intervals (0, 15th, 30th, 45th and 60th day of the experiment) were analyzed for its composition such as total solids, milk fat, solids not fat (SNF), milk protein and milk urea nitrogen (MUN). Four per cent fat corrected milk (FCM) yield, total yield of fat and protein were also calculated. The blood samples were collected at 0, 30th and

60th day of the experiment and were analysed for hemoglobin, plasma glucose, plasma urea nitrogen and plasma calcium and phosphorus. Rumen liquor was collected towards the end of the experiment for the determination of ruminal pH, ammonia nitrogen (NH_3-N) and total volatile fatty acids (TVFA). Body weight of animals was recorded at the beginning and end of the experiment. A digestibility trial was conducted towards the end of the experiment with a collection period of seven days.

Average body weight of experimental animals of the three treatment groups recorded at the 60th day of the experiment was 358.00, 389.50 and 354.50 kg, respectively. There was no significant difference ($P>0.05$) in the body weight of experimental animals recorded at the 60th day of the experiment among the three treatment groups. The average daily dry matter intake (DMI) of animals in the three groups were 11.64, 12.11 and 12.10 kg, respectively and the DMI were 3.25, 3.11 and 3.41 per cent body weight, respectively. There was no significant difference ($P>0.05$) for DMI of animals maintained under the three dietary treatments T1, T2 and T3.

The average daily milk production of experimental animals maintained on dietary treatments T1, T2 and T3 was 8.03, 10.14 and 11.09 kg, respectively. The daily milk production was significantly improved ($P<0.05$) in the brewery waste supplemented (T2) and brewery waste incorporated paddy straw (T3) fed groups when compared with that of control (T1) from fourth week onwards. The milk composition parameters such as percentage of total solids, milk fat, solids not fat (SNF), milk urea nitrogen (MUN) and milk protein remained similar in all the three treatment groups throughout the experiment. The MUN concentration was significantly higher ($P<0.05$) in T2 on 60th day of milk collection than T1 and T3. Brewery waste inclusion had no effect on four per cent FCM yield, yield of milk fat and milk protein content.

Data on blood parameters such as hemoglobin, plasma glucose, plasma urea nitrogen (PUN), plasma calcium and phosphorus did not show any significant difference among the three groups during the 0 and 30th day of collection, while there was significantly higher ($P<0.05$) PUN concentration in T2 than that of T1 and T3.

The average rumen fermentation parameters such as ruminal pH (6.96, 6.75 and 6.87), NH_3-N (24.88, 27.85 and 27.34 mg per 100 ml) and TVFA (101.31, 99.44 and 94.63 meq/l) estimated at the end of feeding trial did not vary significantly ($P>0.05$) among the different treatment groups T1, T2 and T3, respectively.

There was no significant difference ($P>0.05$) in apparent digestibility of DM, OM, CP, EE, NFE, ADF, cellulose and gross energy among the three experimental rations T1, T2 and T3 and respective values were 56.43, 57.90 and 54.94 per cent for DM; 59.12, 60.75 and 58.34 per cent for OM; 60.13, 51.71 and 52.62 per cent for CP; 53.28, 51.69 and 56.63 per cent for EE; 65.50, 66.78 and 65.52 per cent for NFE; 39.10, 43.53 and 40.83 per cent for ADF; 36.95, 38.65 and 40.03 per cent for cellulose and 56.16, 57.59 and 54.60 per cent for gross energy.

Significantly higher apparent digestibility of CF and NDF ($P<0.05$) and hemicellulose ($P<0.01$) was recorded in animals fed T2 and T3 ration than those fed control ration. The digestibility of CF was 50.27, 57.48 and 56.34 per cent, respectively for the three groups. The digestibility of NDF being 47.31, 54.52 and 55.63 per cent and of hemicellulose was 36.85, 44.64 and 46.17 per cent, respectively for the three rations.

The total milk produced in 60 days by the animals fed three dietary treatments T1, T2 and T3 was 2889, 3651 and 3591 kg, respectively. The milk production was higher in brewery waste fed groups (T2 and T3) than that of control (T1). The cost of concentrate mixtures for T1, T2 and T3 were Rs.11.07, 10.38 and 10.38 per kg, respectively. The cost of feed per kg milk produced was Rs.10.40, 7.89 and 8.37 respectively, for the three dietary groups T1, T2 and T3. The cost of production was lower in animals maintained on T2 and T3 rations than that of control.

An *in vitro* experiment was conducted to study the disappearance of DM, CP, CF, NDF, ADF, hemicellulose and cellulose of the following seven experimental feeds such as 1) Control feed; 2) Experimental feed (75%) + Fresh brewery waste (25%) (EFFBW); 3) Experimental feed (75%) + Dried brewery waste (25%) (EFDBW); 4) Fresh brewery waste (FBW); 5) Dried brewery waste (DBW); 6) Paddy straw (PS) and 7) Brewery waste incorporated paddy straw (BWIPS) incubated for 0, 2, 6, 12, 24, 48 and 72 hours in RUSITEC.

In vitro rumen fermentation parameters such as pH, total gas production, CO_2 production, NH_3-N, TVFA and their fractions such as acetate, propionate, iso butyrate, butyrate and valerate concentration and the *in vitro* rumen effective degradability of DM and CP of experimental feeds were studied in RUSITEC.

Among the feeds experimented in RUSITEC, disappearance was more in the control feed, EFFBW and EFDBW when compared to other experimental feeds. Similarly, the disappearance was more in BWIPS compared to paddy straw. The *in vitro* DM, CP, CF, NDF, ADF hemicellulose and cellulose disappearance at all incubation periods were higher for FBW than the DBW. The FBW had more disappearance compared to DBW.

The control feed, EFFBW and EFDBW had higher '*a*' and '*b*' fractions, rate of degradation '*c*' and *in vitro* effective degradability of DM and CP than other experimental feeds such as FBW, DBW, paddy straw and BWIPS. Paddy straw showed lowest *in vitro* '*a*' and '*b*' fractions, rate of degradation '*c*' and effective degradability of DM and CP compared to other feeds experimented in RUSITEC. The BWIPS showed higher *in vitro* effective DM and CP degradability compared to paddy straw. The FBW showed higher DM and CP '*a*' and '*b*' fractions, rate of degradation '*c*' and effective degradability than DBW. The FBW and DBW showed intermediary in '*a*' and '*b*' fractions, rate of degradation '*c*' and *in vitro* effective degradability of DM and CP compared to other experimental feeds in RUSITEC.

A low *in vitro* DM and CP disappearance of 33.24 and 30.75 per cent, respectively was recorded for paddy straw at 72 hours of incubation (effective degradability of 30.59 and 28.64 per cent, respectively for DM and CP), whereas improved *in vitro* DM and CP disappearance of 42.25 and 38.36 per cent, respectively was recorded for BWIPS at 72 hours of incubation (effective DM and CP degradability of 39.93 and 36.32 per cent, respectively).

During initial incubation hours (2, 6, 12 and 24 hours), the control feed, EFFBW, EFDBW and FBW showed higher *in vitro* gas production than other experimental feeds such as DBW, PS and BWIPS. During later incubation hours (48 and 72 hours), DBW, PS and BWIPS showed higher *in vitro* gas production than other experimental feeds. Almost similar *in vitro* carbon dioxide (CO_2) production and pH were observed at all periods of incubation in all the seven experimental feeds incubated in RUSITEC.

The FBW showed highest *in vitro* NH_3-N production at all incubation periods, followed by control feed and EFFBW. The paddy straw had the lowest NH_3-N production at all incubation periods compared to other experimental feeds. However, the BWIPS showed slightly higher NH_3-N production at all incubation periods than paddy straw alone. The EFFBW and DBW showed intermediary NH_3-N production at all periods of incubation compared to other experimental feeds in RUSITEC.

The TVFA concentration was comparatively higher in control feed, EFFBW and EFDBW at all periods of incubation than FBW, DBW, paddy straw and BWIPS. The FBW had higher TVFA concentration than DBW from 12 hours of incubation onwards. The EFFBW had higher TVFA concentration during initial incubation periods (2, 6 and 12 hours) than DBW.

During initial incubation periods (2, 6, 12 and 24 hours) all the experimental feeds showed higher acetate concentration than later incubation periods (48 and 72 hours). The EFDBW showed highest acetate, propionate and iso butyrate concentration (349.64, 266.54 and 90.63 mmol/l, respectively) at 24 hours of incubation, compared to other experimental feeds at all incubation periods. Paddy straw and BWIPS showed lower acetate and propionate concentration at all incubation periods. The control feed, EFFBW, EFDBW, FBW and BWIPS showed higher iso butyrate concentration compared to DBW and paddy straw at all incubation periods.

From the RUSITEC experiment, the effective CP degradability of paddy straw, control concentrate mixture and experimental concentrate mixture (containing 25 per cent brewery waste) were 28.64, 53.97 and 52.37 per cent, respectively. The average daily RUP intake of the animals of three experimental groups T1, T2 and T3 calculated using the effective degradability values were 0.68, 0.71 and 0.72 kg, respectively and the values are higher than the NRC (2001) recommended requirement of 0.50 kg RUP per day for early lactating cows producing 15 kg of milk.

An overall evaluation of the results obtained during the present investigation revealed that inclusion of brewery waste at 25 per cent (DM basis) in concentrate mixture in a paddy straw based diet resulted in significantly improved milk production as well as digestibility of crude fibre, NDF and hemicellulose in medium yielding cows in early lactation.

The cost of feed per kg milk produced was reduced by 24 per cent by the incorporation of brewery waste at 25 per cent level in concentrate mixture (DM basis) in early lactating crossbred cows producing about 10 kg milk per day

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EFFECT OF BREWERY WASTE ON NUTRIENT DIGESTIBILITY OF PADDY STRAW BASED DIET IN LACTATING COWS

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ABSTRACT

Two experiments were conducted, one to assess the effect of brewery waste on nutrient digestibility of paddy straw based diet in lactating cows and the other to study the *in vitro* disappearance, degradability and rumen fermentation characteristics of various experimental feeds in Rumen Simulation Technique (RUSITEC).

In animal study, eighteen early lactating cross bred cows within 20 days of lactation were selected and grouped into three as uniform as possible with regard to their age, body weight, parity, previous milk yield and stage of lactation. They were randomly allotted to the three dietary treatments T1, T2 and T3. The control concentrate mixture and paddy straw formed the T1 ration. The animals of T2 and T3 were fed with experimental concentrate mixture containing 25 per cent brewery waste (on DM basis) and paddy straw as roughage. In T2, the brewery waste was fed separately on wet basis, while in T3, the brewery waste to be incorporated in the concentrate mixture was treated with required quantity of paddy straw, three days before feeding. A feeding trial was carried out for a period of 60 days.

There was no significant difference ($P>0.05$) in body weight and average daily dry matter intake (DMI) of experimental animals among the treatment groups T1, T2 and T3. The animals maintained on the three dietary treatments T1, T2 and T3 showed an average daily milk production of 8.03, 10.14 and 11.09 kg, respectively during the 60 day period of the experiment. The milk production was significantly increased ($P<0.05$) from fourth week onwards in brewery waste fed groups (T2 and T3) when compared with control (T1). The cost of feed per kg milk produced was lower in animals maintained on T2 and T3 rations (Rs. 7.89 and 8.37, respectively) than those fed control ration (Rs. 10.40).

The brewery waste feeding did not affect milk composition such as percentage of total solids, milk fat, solids not fat (SNF) and milk protein and milk urea nitrogen (MUN). The four per cent FCM yield and total yield of milk fat and protein remained unaffected by brewery waste incorporation. Animals in all the three groups showed similar blood parameters such as haemoglobin, plasma glucose, plasma urea nitrogen (PUN) and plasma calcium and phosphorus. The MUN and PUN concentrations were significantly higher ($P<0.05$) in T2 at 60th day than that of T1 and T3.

There was no significant difference ($P>0.05$) in rumen fermentation parameters, such as ruminal pH, NH_3-N and TVFA concentration of the animals fed three experimental diets T1, T2 and T3. There was no significant difference ($P>0.05$) in apparent digestibility of DM, OM, CP, EE, NFE, ADF, cellulose and gross energy among the three treatment rations. Brewery waste fed groups (T2 and T3 ration) recorded significantly higher apparent digestibility of CF and NDF ($P<0.05$) and hemicellulose ($P<0.01$) than those fed control diet.

An *in vitro* experiment was conducted to study the disappearance, degradability and rumen fermentation parameters such as pH, total gas production, CO_2 production, NH_3-N and TVFA and their fractions such as acetate, propionate, isobutyrate, butyrate and valerate concentration of experimental feeds incubated at different incubation periods in RUSITEC.

The following experimental feeds were incubated in the RUSITEC, 1) control feed; 2) experimental feed (75%) + fresh brewery waste (25%) (EFFBW); 3) experimental feed (75%) + dried brewery waste (25%) (EFDBW); 4) fresh brewery waste (FBW); 5) dried brewery waste (DBW); 6) paddy straw (PS) and 7) brewery waste incorporated paddy straw (BWIPS).

Among the feeds experimented in RUSITEC, disappearance was more in the control feed, EFFBW and EFDBW when compared to other experimental feeds. Similarly, the disappearance was more in BWIPS compared to paddy straw. The *in vitro* DM, CP, CF, NDF, ADF, hemicellulose and cellulose disappearance rate at all incubation periods were higher for FBW than those for DBW.

The control feed, EFFBW and EFDBW had higher '*a*' and '*b*' fractions, rate of degradation '*c*' and *in vitro* effective degradability of DM and CP than other experimental feeds such as FBW, DBW, PS and BWIPS and paddy straw showed the lowest. The BWIPS showed higher *in vitro* effective DM and CP degradability compared to paddy straw. The FBW showed higher DM and CP '*a*' and '*b*' fractions, rate of degradation '*c*' and effective degradability than DBW.

A low *in vitro* DM and CP disappearance of 33.24 and 30.75 per cent, respectively were recorded at 72 hours of incubation (effective DM and CP degradability of 30.59 and 28.64 per cent, respectively), whereas the BWIPS had improved *in vitro* DM and CP disappearance of 42.25 and 38.36 per cent, respectively, at 72 hours of incubation (effective DM and CP degradability of 39.93 and 36.32 per cent, respectively).

The data on *in vitro* rumen fermentation characteristics indicated that during initial incubation periods (2, 6, 12 and 24 hours), the control feed, EFFBW, EFDBW and FBW showed higher *in vitro* gas production than other experimental feeds, whereas, during later incubation periods (48 and 72 hours), DBW, paddy straw and BWIPS showed higher *in vitro* gas production than other experimental feeds. Almost similar *in vitro* CO_2 production and pH were recorded at all periods of incubation in seven experimental feeds incubated in RUSITEC. The FBW showed the highest *in vitro* NH_3-N production at all incubation periods, followed by control feed and EFFBW. The paddy straw had the lowest NH_3-N production at all incubation periods compared to other experimental feeds. However, the BWIPS showed slightly higher NH_3-N production at all incubation periods than paddy straw. The EFFBW and DBW were intermediary NH_3-N production at all periods of incubation compared to other experimental feeds in RUSITEC.

The TVFA concentration was comparatively higher in control feed, EFFBW and EFDBW at all periods of incubation than the rest of experimental feeds. The FBW had higher TVFA concentration than DBW in later incubation periods (12, 24, 48 and 72 hours). The EFFBW had higher TVFA concentration during initial incubation periods (2, 6 and 12 hours) than DBW, whereas the DBW had higher TVFA concentration during later incubation periods (24, 48 and 72 hours). During initial incubation periods (2, 6, 12 and 24 hours) all the experimental feeds showed higher acetate concentration than later incubation periods (48 and 72 hours). The EFDBW showed the highest acetate, propionate and iso butyrate concentration (349.64, 266.54 and 90.63 mmol/l, respectively) at 24 hours of incubation, compared to other experimental feeds. The paddy straw and BWIPS showed lower acetate and propionate concentration at all incubation periods.

From the RUSITEC experiment, the effective CP degradability of paddy straw, control concentrate mixture and experimental concentrate mixture (containing 25 per cent brewery waste) were 28.64, 53.97 and 52.37 per cent, respectively. The average daily RUP intake of the animals of three experimental groups T1, T2 and T3 calculated using the effective degradability values were 0.68, 0.71 and 0.72 kg, respectively and the values are higher than the NRC (2001) recommended requirement of 0.50 kg RUP per day for early lactating cows producing 15 kg of milk.

Inclusion of brewery waste at 25 per cent (DM basis) in concentrate mixture in a paddy straw based diet resulted in significantly improved milk production as well as digestibility of crude fibre, NDF and hemicellulose in medium yielding cows in early lactation. The cost of feed per kg milk produced was reduced by 24 per cent by incorporation of brewery waste at 25 per cent level in concentrate mixture (DM basis) in early lactating crossbred cows producing about 10 kg milk per day.