EVALUATION OF UROMIN LICK CONTAINING ALTERNATIVE FEED RESOURCES IN BUFFALOES

Thesis

Submitted to Guru Angad Dev Veterinary and Animal Sciences University in partial fulfilment of the requirements for the degree of

MASTER OF VETERINARY SCIENCE
in
ANIMAL NUTRITION
(Minor Subject: Veterinary Biochemistry)

By

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CERTIFICATE – I

This is to certify that the thesis entitled, "Evaluation of uromin lick containing alternative feed resources in buffaloes" **submitted for the degree of** M. V. Sc., **in the subject of** Animal Nutrition **(Minor Subject: Veterinary Biochemistry) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by** Mahipal Choubey (L-2006-V-02-M) **under my supervision and that no part of this thesis has been submitted for any other degree.**

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE - II

This is to certify that the thesis entitled, **"Evaluation of uromin lick containing alternative feed resources in buffaloes"** submitted by **Mahipal Choubey (L-2006-V-02-M)**, to Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, in partial fulfilment of the requirements for the degree of M. V. Sc., in the subject of **Animal Nutrition** (Minor Subject: Veterinary biochemistry) has been approved by the Student's Advisory Committee along with Head of the Department after an oral examination on the same.

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ABSTRACT

The present study was undertaken to formulate and compare uromin lick (UML) containing waste bread (WB) and tomato pomace (TP) with conventional block and to assess the effect of such UMLs on nutrient utilization in buffaloes. Iso-nitrogenous and iso-caloric blocks were prepared in a manually operated block-making machine. The wheat flour from conventional UML was replaced with waste bread and oiled mustard cake with tomato pomace.

The CP and EE content of different UMLs was comparable. The net gas production, digestibility of nutrients and release of ammonia were statistically comparable. A combination of WB and TP (WBTP) resulted in higher production of VFAs and availability of ME. The comparable partitioning factor also revealed that the efficiency of nutrient utilization from all the UMLs was similar.

In-vivo studies were conducted on adult male Murrah buffaloes (20; 5-6 year old of 442.1±6.3 kg BW) randomly distributed into five equal groups were offered either 2 kg conventional concentrate mixture while in the experimental groups, the animals were offered 1 kg concentrate mixture and *ad lib* respective UML supplemented with 5 kg green fodder and 9 kg wheat straw. The daily intake of concentrate mixture was higher ($P<0.05$) in the control group as compared to those offered UMLs. The daily intake of block varied from 1.08 kg (CB) to 1.84 kg (TPB). The higher ($P>0.05$) wheat straw intake in UMLs gps resulted in higher ($P<0.05$) DM intake in experimental groups. Supplementation of UMLs in the diet of experimental animals improved ($P<0.05$) the digestibility of CP in comparison to control group (C). The digestibility of other nutrients in animals supplemented with UMLs was comparable to that of control group. The N intake was higher ($P<0.05$) in the animals offered UMLs. N-excretion through urine was higher ($P<0.05$) in animals offered UMLs. The N retention was higher ($P<0.05$) in animals offered UMLs as compared to control group.

The rumen studies revealed that the efficient utilization of NPN resulted in higher ($P<0.05$) concentration of TCA-N in WBTPB group as compared to other groups. The blood profile of the animals in all the groups was comparable except the blood urea nitrogen (BUN), which was higher ($P<0.05$) in UML fed groups as compared to control group. All the animals gained weight, but the differences were statistically non significant. It was concluded that WB and TP could be incorporated into UMLs without any adverse effect on palatability, nutrient utilization, rumen metabolites or health of animals.

Key words: Waste bread, Tomato pomace, Uromin lick, *In vitro* and *in-vivo* evaluation, Rumen metabolites, Blood profile nutrient availability, buffaloes.

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Signature of Major Advisor Signature of Student

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LIST OF ABBREVIATIONS USED

ADF	:	Acid detergent fibre
ADL	:	Acid detergent lignin
BV	:	Biological value
BW	:	Body weight
CCM	:	Conventional concentrate mixture
CP	:	Crude protein
CTAB	:	Cetyl trimethyl ammonium bromide
DCP	:	Digestible crude protein
DM	:	Dry matter
DOM	:	Digestible organic matter
EDTA	:	Ethylene diamine tetra acetate
ME	:	Metabolizable energy
MJ	:	Mega joule
NDF	:	Neutral detergent fibre
NDFD	:	Neutral detergent fibre digestibility
NPN	:	Non protein nitrogen
OM	:	Organic matter
TOMD	:	True organic matter digestibility
TP	:	Tomato pomace
TVFA	:	Total volatile fatty acids
UML	:	Uromin lick
WB	:	Waste bread
%	:	Percentage
<	:	Less than

CHAPTER-I

INTRODUCTION

Most of the South Asian countries including India are facing the shortage of feedstuffs. Increase in human population and frequent occurrence of natural calamities create a complex situation, which can be alleviated either by improving the efficiency of utilization of existing nutrients or by tapping new non-conventional feed resources.

It is well established that the ruminants can utilize urea. Enrichment of poor quality roughages by urea and other NPN sources improved the nutrient utilization (Bakshi *et al*., 1986 and Mishra *et al., 2006*), improved milk production (Singh *et al.,* 2007) and thus, helped to save 20-25% concentrate (Tripathi and Sharma, 2002; Verma *et al.*, 2006). However, potential toxicity, characterized by muscular tremors, salivation, tetany and often death of animal might arise owing to either accidental overdoses of urea or faulty methods of using urea. Rapid hydrolysis of urea leads to accumulation of ammonia in peripheral blood, thereby causing ammonia toxicity. For efficient utilization of urea by rumen microbes, a readily soluble carbohydrate/energy source should be supplied in diet along with some minerals (Mc Donald *et al.,* 1987). A number of carbohydrate sources such as paper waste, molasses, starch, chemically treated straws and cereals and agro-industrial by products have been used to serve as energy source for effective urea utilization. Molasses and starch are the most suitable and economic source of energy. Efforts have, therefore, been made to reduce the loss of ammonia by various means like decreasing ureolytic activity of rumen microbes either through production of urease immunity (Siddhu *et al*., 1968) or by starch-controlled-urea-product (Deyoe *et al.,* 1968) or by uromol preparation (Chopra *et al.*, 1974; Langar *et al.,* 1982; Kaushal *et al.,* 1983) or cereal urea-based concentrate mixture (Kaushal, 1999) or urea-molasses liquid diet (UMLD) (Khanna and Malik, 1984; Senegar and Mehra, 1999; Verma *et al.*,2000; Gendley *et al.,* 2003; Can *et al.,* 2004).

The main aim was to complex urea with molasses by heating to form “Maillard product”, which was degraded slowly and consistently in the rumen, resulting in efficient utilization of ammonia in the rumen. But there were a few practical problems of storage, transportation, sudden high intake and requirement of special equipment specifically in case of UMLD, which led to an alternate and more suitable form of urea-molasses supplement *i.e.* uromin licks (UML).

The concept of UML was developed in order to provide blend of nutrients to the animals. Besides urea and molasses it contained wheat flour, oiled seed cake, rice bran and mineral mixture etc. The animals lick such licks and meet part of their nutrient requirement. But the demand and cost of conventional energy (starch/wheat flour) and protein (groundnut/mustard cake) supplements have escalated due to dynamic explosion of human population and urbanization. There is a dire need to exploit alternate energy and protein supplements to add to ever depleting feed basket. Waste bread (left over, unsold, fungal infested etc.) available in abundance, is an excellent source of cooked starch (bypass) and protein (gluten) could be used in the diet of ruminants as substitute of conventional cereal grains (Wadhwa *et al.,* 2008). Tomato pomace is another potential feed resource (consisting of tomato peels, seeds and damaged tomatoes) has 20-22% CP and 10-11% EE (on DM basis). It is a good source of lycopene, a pigment that gives colour to meat and is a known antioxidant. Bakshi *et al* (2008) used dried and ground tomato pomace and replaced concentrate mixture up to 100%, in adult male buffaloes. However, till date neither waste bread nor tomato pomace has been used in the formulations of UML. The present study was, therefore, planned with following objectives:

- To formulate UML containing alternate energy and protein supplements like waste bread and tomato pomace and compare these with conventional uromin lick.
- To assess the effect of such UMLs on nutrient utilization in buffaloes

CHAPTER - II

REVIEW OF LITERATURE

Sansoucy (1986) formulated UML, which contained 50% molasses, 10% urea, 5% salt, 10% quick lime and 25% wheat bran, which was then modified by several research workers according to the availability of ingredients in the region. Such licks prepared are highly hygroscopic in nature (Singh *et al*. 1992). Mehra *et al* (1993) used formaldehyde 225 ml and phosphoric acid 45 ml/100 kg lick, to overcome the problem of the hygroscopic nature of UML and concluded that formaldehyde treated UML is not hygroscopic in nature and it may be recommended for practical use.

UML can be prepared by two processes *i.e.* hot process and cold process. Hot process increases cost of manufacture, as it requires heating device while cold process requires no such device. A lick prepared by cold process appeared to be best from cost and quality point of view (Brar and Nanda, 2005). Several binders have been used by various researchers *viz.* cement (Plaizier *et al.,* 1999; Hag *et al*., *2002*), bentonite (Kakkar *et al*. 1993), bentonite and calcite powder (Toppo *et al*. 1999; Garg and Gupta 1991a), bentonite and calcium oxide (De and Singh 2002), cement and calcium oxide (Rafiq *et al.*1996).

Kakkar *et al.* (1990) prepared uromin lick without an inert binder using hot process and revealed that uromin lick can form a scarcity ration and may be used to correct protein and mineral malnutrition of ruminants, which sometimes occurs during lean periods. Its contribution as source of protein and mineral is equally good for growing and non-productive animals.

Calcium oxide (CaO) as binder is preferred in cold process licks as reaction of calcium oxide and water is exothermic and heat generated is utilized for binding process, gelling effect and hardening process. Lick with 40% molasses (in comparison to 50%) level can be safely stored for one year and licks having calcium oxide (CaO) as binder have greater hardness than those containing Magnesium oxide (MgO). So CaO being cheaper

and better binder should be used in urea molasses mineral lick formation (Sihag and Chahal, 1997). Now, India is leader in production of guar gum with its plentiful availability in North-western region including Punjab, guar gum, due to its easy solubility in hot and cold water, stability at wide range of pH (2-10), highly viscous nature and better water holding capacity could also be used as binder in uromin licks @ 2% (Sihag *et al*., 2003a; Sihag *et al*., 2003b; Sihag *et al*., 2004a). In addition to this, UML can be safely stored for a year at all temperatures (15-45 degrees C) with relative humidity (RH) upto 65% without any visible fungal or mould growth (Sihag *et al*., 2003c)

Kakkar and Makkar (1995) compared certain characteristics of UMLs available from india and abroad with those prepared by Punjab Agricultural university (PAU), Ludhiana. Their study revealed that the product advocated by PAU is harder, better in nutritive value and economic too. The use of UMLs has many fold advantages in the form of better digestion, early/regular heat, improved conception rate and increased life time productivity of dairy animals (Makkar and Saijpaul, 1996). Gupta and Malik (1991) offered UMLs containing subabul leaves to buffalo calves and observed no toxicity symptoms of mimosine and tanin during experiment period of 72 days, which may be due to slow and small intake of this component through subabul lick. They concluded that subabul licks can be introduced in scarcity areas and also to economize ruminant feeding schedule.

2.1 UML Vs CONCENTRATE

Studies revealed that uromin lick (UML) supplementation improves straw utilization (Mohini and Gupta, 1993a) and are suitable substitute for concentrate supplement at maintenance level (Srinivas and Gupta, 1995) without affecting rumen microbial activity (Mohini and Gupta, 1993b). UML can successfully replace concentrate mixture at varying levels depending on the situation. One kg conventional concentrate mixture could be safely replaced by the *ad lib*. feeding of uromin lick with average intake of 559-695g/d in buffalo calves, provided energy intake was made good by other feeds (Kakkar *et al.*, 1997)

Supplementing 50% of energy requirements through the concentrates in a straw-based diet along with UML resulted in increased intake of UML and better utilization of dietary nutrients in adult crossbred cattle (Toppo *et al*, 1997). However, Sihag *et al* (2007b) concluded that 25% of concentrate mixture could be saved by supplementing UML *ad lib* along with basal diet to milch buffaloes yielding around 7 litres of milk in their late lactation. Srinivas and Gupta (1997a) concluded that UML could be partially supplemented up to 10% of the concentrate requirement of crossbred cows yielding on an average 14 kg milk /day without any adverse effect on feed intake, nutrient utilization and milk production. The substitution of concentrate mixture with UML at 25% level lead to an increase in net return on milk sale (Srinivas and Verma, 1996).

Supplementation of UML at 400 and 600 g levels exhibited similar results and its inclusion beyond that had no beneficial effect. Hence, 400 g of UML is the optimum level for maximizing utilization of nutrients from wheat straw-based diets to meet maintenance requirements of adult male buffaloes (Verma *et al* 1998).

To avoid wastage of ammonia due to higher solubility of urea, partial replacement of UML with slowly degraded N supplement like fishmeal was more beneficial for lamb production (Rafiq *et al.,* 1996). UML supplementation increased solid and liquid digesta flow rates by 2 folds when supplemented with wheat straw and improvements were comparable with those on concentrate supplementation (Srinivas and Gupta, 1994). Uromin lick could successfully replace wheat bran and positive response was observed in terms of daily milk yield, extended lactation period, shorten dry period and higher calving rate with enhanced birth weight (Saadullah *et al*., 1994). In UML preparation sunflower meal can effectively replace cottonseed meal up to 9% and UML can substitute commercial concentrates in growing water buffaloes fed on basal ration of wheat straw (Mirza *et al*., 2004). Singh *et al* (1999) supplied concentrate mixture and UML over the oak (Quercus semecarpifolia) leaves to pashmina kids and conclude that concentrate mixture or UML can lessen the negative effect of oak leaves on digestibility of nutrients.

2.2 NUTRIENT UTILIZATION

The *ad-lib* UML Supplementation to wheat straw based diets could supply nutrients (over and above) to adequately meet the maintenance requirement of buffaloes (Sihag *et al*, 2007a). Supplementation of UML increased DM intake, nutrient digestibility and nitrogen balances in crossbred cows fed wheat straw (Srinivas *et al.*, 1996). Rafiq *et al* (1996) showed that UML supplementation economized the rearing of lambs under rangeland condition. Hosamani *et al.* (1995) observed that there was no advantage of supplementing urea beyond 15g/animal /d along with UML, and that fermentable nitrogen from UML alone was not sufficient to meet buffalo's requirement when fed on dry fodder. The 18 m old crossbred calves on straw plus UML supplemented diet showed higher straw intake and exhibited positive N, Ca and P balances (Garg and Gupta, 1992a).

Sengar *et al.* (1995) offered UML to male buffaloes in two different forms viz. as lick and straw and as sani (UML dissolved in water and mixed with straw). Result showed that increased digestibility of nutrient and higher nitrogen balance in sani group. Supplementation of untreated groundnut cake (GNC) or 100g of formaldehyde treated GNC supplemented with UML did not show any beneficial effect in adult cattle (Toppo *et al.*, 1999). Monensin, an antimethanogenic ionophore when used with UML or concentrate mixture did not showed any effect on feed intake and digestibility but elevated blood glucose level in adult cattle (De and Singh, 2002).

Sharma *et al*. (2008) conducted trial to asses the nutrient utilization by feeding of different types of urea molasses mineral licks (UMML) in calves. UMML was prepared by hot and cold methods. Cold processed licks contained calcium oxide, sodium bentonite, phosphoric acid and sodium thio-sulphate as binder at 3, 3, 2 and 1%, respectively. The control group was offered calf grower ration, wheat straw and setaria silage, whereas about 33% protein was replaced by hot processed UMML (T_1) and cold processed UMML (T_2), respectively. The digestibility coefficient of CP, CF, EE and NFE was found to be higher in T_2 compared to T_1 and control. They concluded that feeding UMML manufactured with cold process to calves was more beneficial compared to the other two groups.

Garg *et al.* (1990) tried to see the effect of supplementing UML to untreated or ammonia treated paddy straw in buffalo calves and concluded that straw intake was higher in treated straw group while UML intake was higher in untreated straw group resulting into no significant daily weight gain among the groups.

The UML supplementation resulted in higher intake of barley straw. The DM, OM and CP digestibility increased significantly in UML fed groups. So using UML in lambs fed with poor quality forages may decrease body weight losses and prevent sporadical mortalities in winter conditions of Northeast Turkey (Unal *et al.*, 2005). Sudhakar *et al* (2002) revealed from their study that digestibility for DM, CP and CF were higher in buffaloes fed UML supplemented rations in comparison to un-supplemented UML rations, with rice straw/sorghum stover being the chief roughage source. Toppo *et al* (2000) revealed that 15 g urea supplementation is sufficient to maintain the optimum intake of UML with higher CP digestibility and higher positive N balance as compared to control in adult crossbred cattle.

The results of Hosamani *et al* (1998) indicated that wheat straw supplemented with UML was sufficient to meet the maintenance requirements of adult buffaloes and a higher intake of energy did not have any positive effect on intake of wheat straw and UML. Hosamani *et al* (2000) divided 9 fistulated and 9 intact male buffaloes into three equal groups and offered with wheat straw *ad-lib* and UML as a supplement. In addition animals were given concentrate mixture to meet 80, 100 and 120 percent energy of their requirement. The digestibility of CP, EE and CF were higher in-group I compared to group III. Although rumen metabolites were comparable except total volatile fatty acid (TVFA), which was higher higher in-group II as compared to group III. Results revealed that supplementation of more than 80 percent energy had no extra benefit on lick intake and nutrient utilization in buffaloes.

2.3 RUMEN METABOLITES

Synergy in N source and energy availability for bacterial production could be possible by supplementing wheat straw diets with UML. Addition of a natural protein

source in a UML was found to stimulatory for microbial yield (Srinivas and Gupta, 1997b). UMLs supplementation in straw based diet resulted into ammonia production at slower rate and enhanced the TCA-N over control diet (with 1.5% urea in concentrate mixture) in buffaloes (Kaur and Kakkar, 1994).

Hosamani *et al* (2003) revealed that there was higher total volatile fatty acid (TVFA) production and higher efficiency of nutrient utilization but when UML was supplemented with cereal grains like maize followed by barley and jowar green fodder, but no effect on rumen pH, rumen volume and digesta flow rate due to different sources of energy. De and Singh (2003) found that monensin supplementation either with concentrate or UML decreased molar proportion of acetate and butyrate, and increased that of propionate, decreased ammonia nitrogen and total nitrogen. Both UML as well as monensin decreased methanogenic bacterial and protozoal population but did not affect cellulolytic, proteolytic bacterial population and fungal population.

Feeding of UML containing different energy (deoiled rice bran and wheat bran) and protein sources (mustard cake and deoiled sunflower cake) encouraged optimal fermentation and microbial protein synthesis in rumen of buffalo maintained on wheat straw based diet (Sihag *et al.,* 2003a). They found low NH_3-N values in their study indicating NH_3 was constantly being utilized for synthesis of microbial protein in presence of readily fermentable energy provided by molasses in UML. The microbial activity was poor in cattle fed on wheat straw alone and found to be improved with concentrate or UML supplements resulting into significant increase in rate of degradation of NDF, ADF and cellulose in the rumen (Garg and Gupta, 1992b).

Garg and Gupta (1991b) offered wheat straw *ad lib* plus concentrate (group 1) or three different types of UML (groups 2 to 4) to fistulated Sahiwal × Holstein-Friesian calves. The total VFAs in rumen liquor were 81.25, 77.50, 80.50 and 80.50 mM/l, respectively. Results showed that TVFA concentrations and production rates were not affected by concentrate or UML supplementation of wheat straw in adult ruminants. Toppo

et al (2000) concluded that total N, ammonia N and TVFA were significantly affected due to different levels of urea supplementation (@ 0, 15, 30 and 45 g/animal/ day) in addition to UML *ad lib* and 15 g urea supplementation was optimum for fermentation pattern.

Hosamani *et al* (1998) revealed that gradual additional energy supplied through concentrate mixture to murrah buffaloes resulted in a higher intake of protein and energy than the maintenance requirement, whereas wheat straw supplemented with UML was sufficient to meet the maintenance requirements of adult buffaloes. A higher intake of energy did not have any positive effect on intake of wheat straw and UML. The source of protein (mustard cake/sunflower) cake in UML may affect the molar proportions of different VFAs in the rumen liquor of buffaloes (Sihag *et al.*, 2004b). The UML supplementation resulted in increased concentrations of total N and its fractions, except for trichloroacetic acid precipitable N (TCA-N) due to lick feeding in crossbreed cattle. However, it did not influence rumen TVFA levels, pH, fluid volume and digesta flow rate in fistulated cattle (Toppo *et al.*, 1997).

2.4 BLOOD PROFILE

Mohini and Gupta (1993a) observed an increase in total nitrogen, ammonia-N and urea-N contents of blood of UML supplemented animals but these values remained within physiological limits.

Kang *et al* (2007) offered UML to 30 Pluriparous anoestrus buffaloes during summer to evaluate certain biochemical profiles and to understand the process by which UML supplementation help to fertility. Blood glucose, blood urea nitrogen (BUN) and creatinine concentrations were found to be similar as that of control group (10 anoestrus buffaloes). Their results confirmed that improvement of nutrition through supplementary UML is not reflected through changes in plasma glucose and insulin, and UML proved to be a non-toxic feed supplement for buffaloes, as reflected by normal BUN and creatinine concentrations. However Rafiq *et al* (2000) reported that the concentration of urea in licks positively affected milk and serum urea levels of indigenous dairy cows. Cenesz *et al* (2006) observed no differences in the activities of aspartate aminotransferase (AST),

alanine aminotransferase (ALT) and in level of total protein, glucose, triglyceride and total cholesterol but, there was an increase in total lipid content on lambs supplemented with UML.

Sudhan *et al* (2007) conducted a trial on 300 migratory buffaloes in 10 villages of Jammu district for hematological and biochemical profile. Results revealed an increased in Hb, PCV, TEC, blood glucose and total serum proteins, while the blood urea nitrogen was within normal physiological range in buffaloes after feeding of UML. So balancing diet through UML supplementation could play an important role in the maintenance of animal health.

2.5 COST EFFECTIVENESS

The average cost-benefit ratio of feeding UML prepared by the cold process was 1:2.3 during the summer months (Kang, 2002) and 1:2.9 during spring (Randhawa, 2002). A much higher economic gain of 1:4 was recorded following UML supplementation during the last trimester pre-partum (Brar, 2001). Obviously, the economic return will be more appreciable after feeding UML prepared by the cold process than by the hot process because of the lower costs of the former (Malik *et al*., 1997; Brar, 2001). The actual economic returns from UML supplementation are because of the general improved reproductive performance, better utilization nutrients from low grade roughages in the supplemented animal and Increase in milk yield.

CHAPTER – III

MATERIALS AND METHODS

The uromin licks, containing alternate energy supplement like waste bread and energy cum protein supplement like tomato pomace, were prepared and compared with the conventional UML for proximate, cell wall constituents and availability of nutrients. The licks were evaluated by *in vitro* gas production technique and by conducting *in vivo* trial on adult male Murrah buffaloes. Simultaneously their impact on biochemical changes in the rumen was also assessed in rumen fistulated male buffaloes.

3.1 PROCUREMENT OF ALTERNATE ENERGY AND ENERGY CUM PROTEIN SUPPLEMENT

Waste bread was procured from Cremica Industries, Phillaur, was sun dried for 14 hours. The waste bread was got tested for the level of mycotoxins, from the Department of Veterinary Microbiology, GADVASU. The sun dried waste bread had negligible level of mycotoxins. The tomato pomace (containing tomato peels, seeds and damaged tomatoes) was procured free of cost from Nijjar Agro Industries, Amritsar. The tomato pomace was sun dried and finely ground. The waste bread and tomato pomace were analyzed for proximate (AOAC 1990) and cell wall constituent (Robertson and Van Soest, 1981), before their incorporation in the licks.

3.2 PREPARATIONS OF UML:

Iso-nitrogenous and iso-caloric licks were prepared by manipulation of feed ingredients (Table 1) in a manually operated lick-making machine. The required quantity of molasses and urea were weighed and mixed in a 25 kg capacity iron pan. The guar gum was added to the urea-molasses mixture. Guar gum (due to its easy solubility in hot and cold water, stability at wide range of pH (2-10), highly viscous nature and better water holding capacity) was used as binder in uromin licks.

Table 1. Ingredient composition of different uromin licks, g/3kg lick

Ingredients	**CL**	**WBL**	**TPL**	**WBTPL**
Molasses	900	900	900	900

Urea	300	304	315	319
Mustard cake	300	300	-	-
Deoiled rice bran	300	300	300	300
Wheat flour	450	-	450	-
Waste bread	-	450	-	450
Tomato pomace	-	-	300	300
Mineral mixture	450	450	450	450
Calcium oxide	120	120	120	120
Salt	120	115	105	95
Guar gum	60	60	60	60

CL- Conventional lick; WBL- Waste bread lick; TPL- Tomato pomace lick; WBTPL-Waste bread tomato pomace lick

A premix of other ingredients was prepared (CaO was the last ingredient added to this premix) and added to iron pan with rapid stirring. Heat generated at this stage, converted the contents into a semi-solid mass, which was put into rectangular die of lick making machine. Two UMLs were prepared at a time in a manually operated lick-making machine. The solidified UML were taken out of machine after 5 minutes and packed into polythene bag, with proper labeling and manufacturing date.

3.3 *IN VITRO* EVALUATION OF DIFFERENT UMLS

These licks were analyzed for proximate constituents and different cell wall fractions (section 3.4.7). The digestibility of nutrients and availability of ME from different UMLs was assessed by *in vitro* gas production technique (Menke *et al.*, 1979; Menke and Steingass, 1988).

3.3.1 Preparation of samples

About 375 mg of test material was weighed in a weighing boat (with removable stem) and put at the bottom of the 100 ml calibrated glass syringe taking caution that it should not stick to the walls of syringe. Then the piston, greased with petroleum jelly (Vaseline) was pushed into the cylinder. The syringes containing sample in triplicate were kept in an incubator at 39°C.

3.3.2 Collection of rumen liquor

Rumen fistulated male buffaloes, maintained on 2 kg conventional concentrate mixture (maize 30, mustard cake 10, solvent extracted mustard cake 20, rice bran 15, solvent extracted rice bran 22, mineral mixture 2 and common salt 1 per cent each) 2 kg green and *ad lib* wheat straw, were used for collection of rumen liquor. The rumen contents were collected at 0 hr in double walled (Thermos) flask flushed with CO_2 and maintained at 39°C. The rumen contents were blended for 2-3 min. in a blender, maintained at 39°C and then strained through 4 layered muslin cloth.

3.3.3 Preparation of solutions

Following solutions were prepared well in advance

1) Micro mineral solution

$CaCl_2.2H_2O$ =13.2 g

$MnCl_2.4H_2O$ = 10.2 g

$CoCl_2.6H_2O$ = 1 g

$FeCl_3.6H_2O$ = 8 g

Dissolved in distilled water and made the volume 100 ml.

2) Macro-mineral solution

Na_2HPO_4 = 5.7 g

KH_2PO_4 = 6.2 g

$MgSO_4$.7H2O = 0.6 g

Dissolved in distilled water and made the volume 1000 ml.

3) Buffer solution

$NaHCO_3$ = 35.0 g

NH_4HCO_3 =4.0 g

Dissolved in distilled water and made volume 1000 ml.

4) Resazurine solution: Dissolved 100 mg of resazurine in distilled water and made volume 100 ml and kept in refrigerator.

5) Reducing solution: This solution is to be prepared fresh at the time of use.

$Na_2S.H_2O$ = 373.0 mg

1N NaOH = 2.6 ml

Distilled water =62.0 ml

The above solutions were mixed in following ratio in a Woulff flask (3 litre cap.) mixed with magnetic stirrer in a water bath at 39°C.

1. Distilled water = 960 ml
2. Micro mineral solution = 0.16 ml
3. Buffer = 660 ml
4. Macro mineral solution = 330 ml
5. Resazurine = 1.6 ml

50 ml of reducing solution was added.

3.3.4 Procedure

While the reducing solution was added, CO_2 was flushed through a submerged tube, till the slightly bluish color first turned pinkish then became colorless. The strained rumen liquor (SRL) was added to the buffer media in 1:2 ratios, only when solution was colorless. The flushing of CO_2 was continued till the last syringe was filled.

For filling up of syringes, the tube on the capillary attachment to the syringe was firmly fixed on to the bottle top dispenser. 30 ml of SRL: buffer solution from the flask kept in a water bath was pumped in each syringe. Gentle shaking mixed the contents in syringe. Air bubbles were brought to the surface and removed through the capillary by careful upward movement of the piston. The clip was closed immediately and exact volume of the contents in the syringe was noted and kept in a water bath maintained at 39°C. The contents in all the syringes were swirled at 1-hour interval for first few hours. If at 8h the gas exceeded 70 ml, the volume of gas was recorded and gas was removed. After 24 hours

volume of gas produced in each syringe was recorded. Blanks and standard hay in triplicate were also run with each set of incubation. After stipulated time, the contents were taken out and centrifuged. After 24 hr the NH_3-N, TVFA and NDF of residue were determined. The ME value of the substrate was calculated by using the following equation developed by (Menke *et al*., 1979)

ME = 1.24 + 0.146 G + 0.007 CP + 0.0244 EE

ME = Metabolisable energy, MJ/kg DM

G = Net gas production, ml/200 mg DM

CP = Crude protein, g/kg DM

EE = Ether extract, g/kg DM

After the *in vitro* experiments, the lick prepared were offered to adult male Murrah buffaloes to evaluate the effect of different UMLs on utilization of nutrients.

3.4 *IN VIVO* EVALUATION OF COMPLETE FEED SUPPLIMENTED WITH OR WITHOUT UML

3.4.1 Selection, distribution and feeding regimen of animals

Adult male Murrah buffaloes (20; 5-6 yr old of 442.1±6.3 kg BW) were randomly distributed into five equal groups. The animals in the control group (C) were fed 2 kg conventional concentrate mixture (maize 30, mustard cake 10, solvent extracted mustard cake 20, rice bran 15, solvent extracted rice bran 22, mineral mixture 2 and common salt 1 per cent each) while in the experimental groups, the animals were offered 1 kg concentrate mixture and UML *ad lib* supplemented with 5 kg green fodder and 9 kg wheat straw. The feeding schedule of the animals is presented in Table 2.

Table 2. Feeding schedule of adult male buffaloes, kg/d

S. No.	Group	Wheat straw	Green fodder	Concentrate mixture	UML
1.	Conventional (Control)	9	5	2	-
2.	CL	9	5	1	*ad lib*
3.	WBL	9	5	1	*ad lib*
4.	TPL	9	5	1	*ad lib*
5.	WBTPL	9	5	1	*ad lib*

CL- Conventional lick; WBL-Waste bread lick; TPL- Tomato pomace lick; WBTPL-Waste bread tomato pomace lick

3.4.2 Housing

The animals were housed in a concrete shed and were stall fed individually at 9:00 AM daily. The animals had free access to water twice a day and were taken out in the yard for 1-hour exercise daily.

3.4.3 Weighing of animals

The animals were weighed for 3 consecutive days at 15 days interval before feeding and the feeding schedule was adjusted accordingly.

3.4.4 Metabolism trial and collection of faeces, urine and residues

At the termination of experimental period, a 7-day metabolic trial was conducted on all the animals. During metabolic trial, the animals were kept in specially designed metabolic cages having a cemented floor converging in centre, where a metallic pipe led the excreted urine into a narrow mouth plastic container (25 litre capacity) containing 400 ml of 20% H_2SO_4. The collection of faeces and urine was done for 7 days. The faeces voided were collected manually and kept in metallic drums (30 kg capacity) for 24 h. Faeces were collected manually by trained persons, who were put on duty round the clock. However, the collection of urine was automatic. The combined residue of wheat straw, green and concentrate mixture, if any, was weighed every morning at 9.00 o' clock in the morning before offering the next day's ration. On the last day of trial, blood sample was taken from jugular vein of animals 4 hrs. of post feeding to determine related blood profile of animals.

3.4.5 Sampling of feed, orts, faeces and urine

3.4.5.1 Feed

Samples of feedstuffs and orts were collected at 24 h interval and dried in duplicate at 100^{o} C in a forced air oven. The samples were pooled for 7 days, finely ground to pass through 1mm sieve and analyzed for proximate principles and cell wall contents.

3.4.5.2 Faeces

After thoroughly mixing, the 24 h faecal material of each animal, one by two hundredth parts in duplicate was weighed in circular aluminum tray for DM determination. The samples were dried at 100^{o} C in a forced air oven overnight and weighed by taking minimum time to prevent the absorption of moisture from atmosphere. The 7 days dried faeces were pooled, and finely ground to pass through 1mm sieve and preserved in airtight glass sampling bottles. For nitrogen estimation one by four hundredth part of total faeces was preserved in previously tarred wide mouth plastic bottles daily, containing 25 ml of 20% sulphuric acid solution added on the first day of collection. Everyday, after placing the faeces in the bottles, it was thoroughly mixed to prevent loss of ammonia and possible infestation with fungi. Faecal samples were stored in a refrigerator till analyzed.

3.4.5.3 Urine

An aliquot of urine equal to one by hundred part of the total urine voided was preserved in narrow mouth glass bottles (1000 ml capacity) daily, which were kept in a refrigerator till analyzed for nitrogen content

3.4.6 Analysis of feeds, faeces and urine

Samples of different UMLs, concentrate mixture, wheat straw, green fodder, feed residue and faeces were analyzed for their proximate constituents (AOAC, 1990) and cell wall constituents (Robertson and Van Soest, 1981).

3.4.7 Proximate principles

3.4.7.1 Dry matter

For this, a known quantity of the well-mixed sample was taken in an aluminum tray, and then it was dried in an oven at 100^{o} C for 24 h. The weight of the dried sample was taken as the dry matter content of the sample.

3.4.7.2 Total ash

Finally ground sample (2 gm) was taken in duplicate in tarred crucibles over a hot plate and then ignited in muffle furnace for 3 hours. After that the crucibles were taken out put in desiccators and weighed. The difference between initial weight of empty crucible and final weight of crucible with ash gave the total ash content in the sample and was expressed as percent of DM (AOAC 1990). The loss in weight after ignition in muffle furnace is taken as organic matter.

3.4.7.3 Crude protein

The N content was estimated by Macro-Kjeldahl method (AOAC, 1990). Finely ground sample (0.25 g) or 2 ml of urine or 5 gm of fresh feaces was digested with 15 ml (25 ml for feaces) of concentrated sulphuric acid and (5-6 gm of digestion mixture potassium sulphate and copper sulphate, 9:1). The material after digestion was distilled in presence of 40 per cent sodium hydroxide. The ammonia liberated was collected in 20 ml of 4 per cent of boric acid-mixed indicator solution. Mixed indicator was prepared by taking bromocresol green and methyl red in 5:1 ratio in 95% ethanol. Ammonium borate thus formed was titrated against standard (0.1 N) sulphuric acid. The reading for blank was also recorded.

$$\text{Nitrogen (\%)} = \frac{(\text{Vol. of acid used} \times \text{Normality of acid} \times 0.014)}{\text{Wt. of sample (g)}} \times 100$$

The CP (%) content was calculated by multiplying the nitrogen by 6.25.

3.4.7.4 Ether extract

Finely ground (2 gm) sample was transferred to extraction thimble (Whatman No.1). The sample was extracted in Soxhlet apparatus with petroleum ether (60-80°C) for 16 hr. After 16 hr the excess of petroleum ether was collected from the Soxhlet apparatus and small volume left in flask at the bottom is transferred to tarred 100 ml beaker. The beaker was dried in an oven to a constant weight. The difference in initial (weight of empty beaker) and final weight (beaker with ether extract) gave the ether extract content in the sample. The ether extract was expressed as per cent ether extract on DM basis (AOAC, 1990).

3.4.8 Cell wall constituents

3.4.8.1 Acid detergent fiber

The test sample (1g) was transferred in spout less beaker and 100 ml of acid detergent solution was added (20 g TAB dissolved in one litre of 1N H_2SO_4). The contents were refluxed for one hour. The contents were filtered through previously weighed sintered glass crucibles (G-1) and washed with hot water till free from ADS followed by one washing of acetone. The residue was dried at 80°C in a hot air oven for overnight. The difference in initial (empty crucible) and final (crucible + residue) weight of crucible gave ADF content. It was expressed as per cent on DM basis (Robertson and Van Soest, 1981).

3.4.8.2 Neutral detergent fiber

Finely ground sample (0.5 gm) was transferred in spout less beaker and 50 ml of neutral detergent solution was added. The NDS was prepared as follows 18.61 gm disodium salt of EDTA and 6.81 gm sodium borate were taken in a beaker, added some distilled water and heated till dissolved. Then 4.56 gm disodium hydrogen orthophosphate was taken in another beaker, added distilled water and heated till dissolved. Then, added 30 gm sodium lauryl sulphate and 10 ml of ethoxy ethanol in 850 ml distilled water. Then added contents of two previous beakers to it and mixed. The volume was made to one litre.

The sample and neutral detergent solution were refluxed for one hour, after the boiling had started. The contents were filtered through previously tarred sintered glass crucible (G-1) and washed with hot water, till free from NDS, followed by final washing with acetone. The residue was dried at 80°C in a hot air oven for overnight. The difference in initial weight (empty crucible) and final (crucible + residue) weight of crucible gave the NDF content. It was expressed as per cent NDF on DM basis (Robertson and Van Soest, 1981).

3.4.8.3 Cellulose

Half g sample was taken and 15 ml of digestion mixture solution (650 ml glacial acetic acid, 80 ml nitric acid and 150 ml distilled water) was added in plastic tubes. The tubes were placed in boiling water bath for 30 minutes. Then contents were filtered through sintered glass crucible (G-1), washed with hot water repeatedly and then final washing with ethanol. The residue was dried at 80°C for overnight in a hot air oven. Then crucibles were

weighed and ignited at 500°C in a muffle furnace for half an hour. The loss in weight on ignition represents cellulose content, which was expressed as per cent cellulose on DM basis (Crampton and Maynard, 1938).

3.4.8.4 Acid detergent lignin (ADL)

Sulphuric acid (72%, w/v) was added to the sintered crucibles containing ADF and kept at room temperature for 3 hour. After draining the acid, the residue was washed with water till it became acid free, dried in hot air oven for overnight and then weighed. It was then ignited in a muffle furnace at 500°C for 3 hours. The loss in weight upon ignition represented ADL, which was expressed as per cent on DM basis. (Robertson and Van Soest, 1981).

3.5 Rumen studies

In order to see the effect of different UMLs on bio-chemical changes in rumen metabolites, rumen studies were conducted on three adult male Murrah fistulated buffaloes fitted with permanent rumen fistulae. One diet was tested at a time on all the 3 fistulated animals.

3.5.1 Feeding schedule

The ration of animal under control group consisted of 2.0 kg concentrate mixture, 9.0 kg wheat straw and 5.0 kg of green per day. In experimental groups only 1.0 kg concentrate mixture was offered/animal/day and supplemented with either *ad lib* conventional lick, waste bread lick, tomato pomace lick or waste bread-tomato pomace lick.

3.5.2. Collection and analysis of rumen liquor samples

After 30 days adaptation on a particular ration, the rumen liquor samples form each animal were collected for 2 consecutive days at 2 hourly intervals, starting form zero and continuing up to 10 hour post-feeding. The rumen liquor samples were strained through four layer of muslin cloth and few drops of saturated mercuric chloride solution were added to arrest the microbial activity. The samples of rumen liquor were pooled for the respective animal and the pH was measured immediately by using a digital pH meter

and the samples were stored in a refrigerator till analyzed for the various N fractions and the total VFA contents. On 3rd day, 50g polyethylene glycol (PEG) molecular weight 4000 dissolved in 100ml water was introduced in the rumen and rumen liquor was collected as per above schedule. The SRL samples were analyzed for PEG (Hyden, 1955; Russell, 1982) to measure liquid outflow parameters.

3.5.3 Total nitrogen

A 5 ml aliquot from each of the SRL samples was taken in kjeldhal flask and added 10 ml conc. sulfuric acid along with 2 gm digestion mixture (potassium sulfate: copper sulfate:: 9 : 1). Kjeldhal flask was heated for digestion till the samples become clear light green. The digested and cooled samples were transferred to distillation flask with 250 ml distilled water, and an access of 40% NaOH (40 ml) was added to release ammonia from ammonium sulfate formed during digestion. The ammonia liberated was absorbed in 25 ml of 4% boric acid solution having mixed indicator. The formed ammonium borate was titrated against N/10 H_2SO_4. The % N was calculated after subtracting the blank.

$$\text{Nitrogen (\%)} = \frac{(\text{Vol. of acid used} \times \text{Normality of acid} \times 0.014)}{\text{SRL (ml)}} \times 100$$

3.5.4 TCA- ppt nitrogen

A 5 ml aliquot from each of SRL was taken in centrifuge tube and 5 ml of 20 % tri-chloroacetic acid (TCA) was added and left for overnight. It was centrifuged at 2000 rpm for 15 minutes. Sediment was transferred by repeated washing in kjeldhal flask. The digestion, distillation and titration was done as total nitrogen estimation:-

$$\text{TCA ppt. N (\%)} = \frac{(\text{Vol. of acid used} \times \text{Normality of acid} \times 0.014)}{\text{SRL (ml)}} \times 100$$

3.5.5 Non-protein nitrogen (NPN)

Non-protein nitrogen = Total nitrogen – TCA ppt. nitrogen

3.5.6 Ammonical nitrogen

A 2 ml of aliquot from each SRL was taken in one litre distillation flask containing 250 ml of water and distilled in presence of 2 ml of NaOH. The ammonia liberated was collected in 20 ml of 4 per cent boric acid solution containing mixed indicator (same as in case of nitrogen estimation). Ammonium borate thus formed was titrated against standard 1/100 N sulphuric acid. The per cent ammonical nitrogen was calculated as follows after subtracting the blank (AOAC, 1990).

$$\text{Ammonical nitrogen (\%)} = \frac{(\text{Vol. of acid used} \times \text{Normality of acid} \times 0.014)}{\text{sample (ml)}} \times 100$$

3.5.7 Total volatile fatty acids (TVFA's)

The total volatile fatty acids were estimated by the method of Barnett and Reid (1957). The boiling flask of 'Markham' distillation apparatus was filled with distilled water and apparatus was steam washed for 10 min. then 1 ml of SRL and 1 ml of buffer solution (mixture of 5 % oxalic acid and 10 % potassium oxalate in 1:1 ratio) was added through the inlet into the steam distillation compartment of 'Markham' apparatus. After washing, the inlet was filled with water to ensure proper sealing. About 100 ml of distillate was collected and titrated against N/100 NaOH, using phenolphthalein as indicator. After subtracting the blank, the value is expressed as milliequivalent of TVFA per ml.

3.6 Blood profile

The blood sample was collected (in heparin and sodium flouride + oxalate vials) from the juglar vein of animals at 4 h post parandial. The serum was separated and stored at 0^{o} C till analyzed. The analysis was conducted on Erba (Mannheim) Chem 5X (Transasia). The serum collected with sodium fluoride and oxalate was used for assay of blood glucose (Trinder, 1969).

3.6.1 Blood glucose

It was estimated by Glucose Oxidase/Peroxidase method using Bayer's diagnostic kit (Trinder, 1969).

3.6.1.1 Reagents:

Reagent 1 (Buffer/Enzymes/Chromogen):

Phosphate Buffer	95 mmol/L
4-Aminoantipyrine	0.2 mmol/L
p-Hydroxy Benzoic Acid	5.9 mmol/L
Glucose Oxidase	$\geq$ 5000 U/L
Peroxidase	$\geq$ 5000 U/L

Standard (Glucose 100 mg/dL):

Glucose	1 g/L

3.6.1.2 Reagent reconstitution

Gently dissolve 1 tablet in 50 ml of distilled water (for pack size of 50×50 ml) in a clean beaker with continuous stirring. Transfer the solution into dark bottle and label this solution as "working solution".

3.6.1.3 Procedure

To 10 µl distilled water, standard and serum, 1 ml of reagent was added. The mixture was mixed thoroughly and left for 15 minutes at 37° C temperature. Concentration was noted by using Erba (Mannheim) Chem 5X (Transasia) at 500nm.

Serum with heparin was used for analysis of total protein, albumin, urea, Ca and P as follows.

3.6.2 Total protein

It was estimated by Biuret method using Bayer's diagnostic kit (Henry *et al*, 1974).

3.6.2.1 Reagents:

Reagent 1 (Biuret Reagent):

Sodium Hydroxide	3.8 mol/l
Potassium Sodium Tartarate	0.1 mol/l
Cupric Sulphate	33 mmol/l
Potassium Iodide	30 mmol/l

Reagent 1A (Surfactant):

Surfactant	20 g/l
Standard (Total Protein g/dL):	
BSA	60 g/l

3.6.2.2 Reagent reconstitution

Allow the reagent to attain room temperature.

Add 41 ml of distilled to one bottle of reagent 1 and then mixed the contents with one bottle of reagent 1A.

3.6.2.3 Procedure

To 10 µL distilled water, standard and serum, 1 mL of reagent was added. The contents were mixed thoroughly and left for 20 minutes at room temperature. Concentration was noted by using Erba (Mannheim) Chem 5X (Transasia) at 500nm.

3.6.3 Albumin

It was estimated by Bromocresol Green (BCG) method using Bayer's diagnostic kit (Doumas *et al.*, 1971).

3.6.3.1 Reagents:

Reagent 1 (Bromocresol Green):	
Succinic acid	94 mmol/l
Sodium Hydroxide	10.2 mmol/l
BCG	0.149 mmol/l
Standard (Albumin 5 g/dl):	
BSA	50 g/l

3.6.3.2 Reagent reconstitution

Albumin reagent is ready to use.

3.6.3.3 Procedure

To 10 µL distilled water, standard and serum, 1 ml of reagent was added. The contents were mixed thoroughly and left for 1 minute at room temperature. Concentration was noted by using Erba (Mannheim) Chem 5X (Transasia) at 628 nm.

3.6.4 Globulin

Globulin was calculated by difference as follows:

Globulin = Total protein – Albumin

3.6.5 Urea

It was estimated by urease method using Bayer's diagnostic kit (Evans, 1968).

3.6.5.1 Reagents:

Reagent 1 (Urease)	
Urease	>1 KSU/l
Reagent1A (Buffer):	
Disodium EDTA	0.1 mol/l
Sodium Nitrprusside	6 mmol/l
Reagent 2 (Phenol):	
Phenol	1.8 mmol/l
Reagent 3 (Hypochlorite):	
Sodium Hypochlorite	0.47 mol/l
Standard (Urea 40 mg/dl):	
Urea	0.4 g/l

3.6.5.2 Reagent reconstitution

Allow the reagent to attain room temperature.

Solution (1)

Transfer the contents of one bottle of reagent 1A into one bottle of reagent 1. Mix gently.

Solution (2)

Add 77 ml of distilled water into one bottle of reagent 2. Mix gently.

Solution (3)

Add 77 mL of distilled water into one bottle of reagent 3. Mix gently.

3.6.5.3 Procedure

To distilled water, standard and serum, 100 µl of solution 1 was added and left for 10 minutes at 37°C, followed by addition of 1.5 ml each of solution 2 and 3. The contents

were mixed thoroughly. Concentration was noted by using Erba (Mannheim) Chem 5X (Transasia) at 546 nm after 15 minutes.

3.6.6 Calcium

It was estimated by Cresolphthalein Complexone method using Bayer's diagnostic kit (Henry and Dryer, 1963).

3.6.6.1 Reagents:

Reagent1 (Cresolphthalein Complexone):	
Dimethyl Sulfoxide	1.4 mol/l
8-Hydroxyquinoline	17 mmol/l
Cresolphthalein Complexone	0.06 mmol/l
Reagent 2 (Buffer):	
Potassium Cyanide	7.6 mmol/l
Diethyl Amine	0.38 mol/l
Standard (Calcium 10 mg/dl):	
Calcium	0.1 g/l

3.6.6.2 Reagent reconstitution

Allow the reagent to attain room temperature.

The working solution was prepared fresh before use. Equal volumes of reagent 1 and reagent 2 were mixed to obtain working solution.

3.6.6.3 Procedure

To 10 µl distilled water, standard and serum, 1 ml of reagent was added. The contents were mixed thoroughly and left for 5 minutes at room temperature. Concentration was noted by using Erba (Mannheim) Chem 5X (Transasia) at 575nm.

3.6.7 Phosphorus

It was estimated by UV Endpoint method using Bayer's diagnostic kit (Amador and Urban, 1972).

3.6.7.1 Reagents:

Reagent 1 (Ammonium Molybdate):

Sulphuric Acid	1.4 mol/l
Ammonium Molybdate	3.2 mmol/l
Reagent2 (Surfactant):	
Surfactant	6 g/l
Standard (Phosphorus 4 mg/dL):	
Inorganic Phosphorus	40 g/l

3.6.7.2 Reagent reconstitution

Allow the reagent to attain room temperature.

The working solution was prepared fresh before use. Equal volumes of reagent 1 and reagent 2 were mixed to obtain working solution.

3.6.7.3 Procedure

To 10 µl distilled water, standard and serum, 1 ml of reagent was added. The contents were mixed thoroughly and left for 5 minutes at room temperature. Concentration was noted by using Erba (Mannheim) Chem 5X (Transasia) at 340 nm.

3.6.8 Triglycerides

It was estimated by enzymatic colorimetric method using Bayer's diagnostic kit (Eggstein and Kuhimann 1974).

3.6.8.1 Reagents:

Reagent 1 (Enzymes/Chromogen):	
Lipoprotein Lipase	$\geq$ 1100 U/l
Glycerol Kinase	$\geq$ 800 U/l
Glycerol -3- Phosphate Oxidase	$\geq$ 5000 U/l
Peroxidase	$\geq$ 1100 U/l
4-Aminoantipyrine	0.7 mmol/l
ATP	0.3 mmol/l
Reagent 1 A (Buffer):	
Pipes buffer, pH 7.5	50 mmol/l
ADPD	1 mmol/l

Magnesium salt	15 mmol/l
Standard (Tryglycerides 200 mg/dL):	
Glycerol (Trig. Equivalent)	2 g/l

3.6.8.2 Reagent reconstitution

Allow the reagent to attain room temperature.

Dissolve the contents of one bottle of reagent 1 with one bottle of reagent 1 A. Mix by gentle swirling.

3.6.8.3 Procedure

To 10 µl distilled water, standard and serum, 1 ml of reagent was added. The contents were mixed thoroughly and left for 5 minutes at room temperature. Concentration was noted by using Erba (Mannheim) Chem 5X (Transasia) at 546 nm.

3.6.9 Cholesterol

It was estimated by enzymatic method using Bayer's diagnostic kit (Allain *et al* 1974).

3.6.9.1 Reagents:

Reagent 1 (Enzymes/Chromogen):	
Cholesterol Esterase	$\geq$ 200 U/l
Cholesterol Oxidase	$\geq$ 250 U/l
Peroxidase	$\geq$ 1000 U/l
4-Aminoantipyrine	0.5 mmol/l
Reagent 1 A (Buffer):	
Pipes buffer, pH 6.90	50 mmol/l
Phenol	24 mmol/l
Sodium Cholate	0.5 mmol/l
Standard (Cholesterol 200 mg/dL):	
Cholesterol	2 g/l

3.6.9.2 Reagent reconstitution

Allow the reagent to attain room temperature.

Dissolve the contents of one bottle of reagent 1 with one bottle of reagent 1 A. Mix by gentle swirling.

3.6.9.3 Procedure

To 10 µl distilled water, standard and serum, 1 ml of reagent was added. The contents were mixed thoroughly and left for 5 minutes at room temperature. Concentration was noted by using Erba (Mannheim) Chem 5X (Transasia) at 500 nm.

3.6.10 Creatinine

It was estimated by picrate method using Bayer's diagnostic kit (Henry *et al* 1974).

3.6.10.1 Reagents:

Reagent 1 (Picrate):

Picric Acid	34.9 mmol/l
Sodium Hydroxide	45 mmol/l

Reagent 2 (Sodium Hydroxide):

Sodium Hydroxide	0.26 mol/l

Standard (Creatinine 2 mg/dL):

Creatinine	0.020 g/l

3.6.10.2 Reagent reconstitution

Allow the reagent to attain room temperature.

Mix equal volumes of reagent 1 and reagent 2 in a clean beaker. The working solution is stable for 3 days at 15^{o} C-25^{o} C.

3.6.10.3 Procedure

To 1 ml Working solution, standard and serum, 100 µl of reagent was added. The contents were mixed thoroughly and left for 5 minutes at room temperature. Concentration was noted by using Erba (Mannheim) Chem 5X (Transasia) at 500 nm.

3.7 Statistical analysis

The data of chemical composition, *in vitro* evaluation and *in vivo* studies were evaluated by simple ANOVA (Snedecor and Cochran, 2004) by using SPSS ver. 12 and means were compared by using Tukey b.

CHAPTER-IV

RESULTS AND DISCUSSION

The uromin licks, containing waste bread and/or tomato pomace were prepared and compared with the conventional UML for chemical composition and availability of nutrients. The licks were evaluated by *in vitro* gas production technique and *in vivo* study on adult male Murrah buffaloes. Simultaneously their impact on biochemical changes in the rumen was also assessed in rumen fistulated male buffaloes. The results obtained and their interpretations have been reported under following heads:

4.1 CHEMICAL COMPOSITION OF WASTE BREAD AND TOMATO POMACE

The chemical analysis revealed that the waste bread contained 12.5 % CP (Table 3). The chemical composition of waste bread was quite comparable to the one reported earlier (Wadhwa *et al.,* 2008; Bhargava, 2008). The composition of waste bread was comparable to that of wheat grains (Kaur *et al*, 2006; Bhargava, 2008). The dried tomato pomace proved to be an excellent source of both proteins (20.9 %) and fat (11.0 %), confirming earlier report (Bakshi *et al.,* 2008). Besides energy and protein, tomato pomace is a good source of phosphorus, essential fatty acids (linoleic acid), lysine, vitamin E and lycopene; a pigment which gives a typical colour to meat and acts as an antioxidant (Wenli et al., 2001; Kravchenko et al.,2003)

Table 3. Chemical composition of waste bread and tomato pomace, % DM

Constituent	Waste bread	Tomato pomace
OM	97.2	93.1
CP	12.5	20.9
EE	1.3	11.0
NDF	10.0	68.0

ADF	2.0	53.0
Cellulose	1.5	38.0
Hemi-cellulose	8.0	15.0

The CP content of tomato pomace was higher than that in cereal grain (9-11 %) but less than that in conventional oil seed cakes (32-36%). The cell wall constituent i.e. NDF, ADF, cellulose and hemi cellulose were much higher in tomato pomace as compared to that in waste bread. Keeping these points in mind, wheat flour from conventional UML was replaced with waste bread and oiled mustard cake with tomato pomace.

4.2 CHEMICAL COMPOSITION OF FEEDSTUFFS

The chemical composition of wheat straw, green, concentrate mixture and that of four different UMLs, prepared by using different combinations of waste bread and tomato pomace has been presented in Table 4.

Table 4. Chemical composition of different feedstuffs, % DM basis

Const.	Concentrate	CL	WBL	TPL	WBTPL	Green	WS
Total ash	9.5	27.6	27.2	27.1	26.5	13.2	7.5
OM	90.5	72.4	72.8	72.9	73.5	86.8	92.5
CP	21.4	41.2	41.4	41.6	41.7	20.8	3.4
EE	4.1	1.4	1.2	1.83	1.83	2.0	1.0
NDF	30.0	11.0	10.0	13.5	12.0	46.0	78.0
ADF	14.0	6.3	6.0	7.5	7.3	35.0	50.5
Cellulose	8.0	2.0	2.0	3.0	2.5	18.0	41.0
HC	15.5	4.8	4.0	6.0	4.8	11.0	27.5

WS- Wheat straw; C- Conventional; WB- Waste bread; TP- Tomato pomace; L- lick

The ingredients used for formulation of licks were proportioned to get isonitrogenous and isocaloric licks (Table 1). The chemical analysis of different UMLs (Table 4) showed that total ash varied between 26.5 to 27.6 % and was comparable amongst all UMLs. The high ash content in the licks could be due to high level (150g *vs* 10g/kg) of mineral mixture used. The CP and EE content of different UMLs was similar indicating that licks were isonitrogenous and isocaloric. The CP content of concentrate mixture and green fodder was almost alike on DM basis as the fodder supply during the trial period was mostly leguminous *i.e.* berseem. The low cell wall constituents in the conventional lick (CL) and the lick containing waste bread (WBL) than that in the licks containing tomato pomace (TPL and WBTPL) was because of higher fiber fraction in tomato pomace. The low cell wall constituents in different licks, as compared to the other feedstuffs was due to use of molasses and concentrate feed ingredient in UMLs.

4.3 *IN VITRO* EVALUATION OF CONVENTIONAL LICK AND LICK CONTAINING WASTE BREAD AND TOMATO POMACE

The *in vitro* evaluation of different licks has been presented in Table 5. In experimental licks wheat flour was replaced with waste bread, while mustard cake was replaced with tomato pomace on nitrogen basis.

The results revealed that the net gas production and digestibility of nutrients (OM and NDF) was similar in all the licks. The *in vitro* release of ammonia during fermentation of nutrient from different licks was also statistically comparable. The total volatile fatty acid production varied from 8.95 meq/dl (TPL) to 9.75 meq/dl (CL). Replacement of cereal grains with WB (WBL) showed no significant effect on the production of TVFA, and level was statistically comparable ($P>0.05$) to that of CL. However, replacement of mustard cake with TP alone (TPL) resulted in depression ($P<0.05$) in TVFAs as compared to those produced from the CL. A combination of WB and TP (WBTP) proved to be a better option as far as production of VFAs and availability of ME was concerned. The availability of ME

(MJ/kg DM) from different licks varied from 5.71 (WBL) to 6.03 (WBTPL). The low ME could be attributed to high total ash content in all licks, which is negatively correlated with

Table 5. *In vitro* utilization of nutrients from different UMLs.

Parameter	CL	WBL	TPL	WBTPL	Pooled SE
NGP, ml/g DM/24h	95.70	94.49	93.64	93.44	0.51
OMD, %	69.87	69.57	69.26	69.45	0.10
NDFD, %	10.39	10.27	10.15	10.39	0.11
PF	3.84	3.89	3.83	3.85	0.03
NH_3-N, %	0.058	0.057	0.057	0.056	0.00
TVFA, meq/dl	9.75^{b}	9.50^{ab}	8.95^{a}	9.50^{ab}	0.12
ME, MJ/kg DM	5.78^{ab}	5.71^{a}	5.89^{b}	6.03^{c}	0.04

NGP- Net gas production; D-Digestibility; PF- Partitioning factor; Figures with different superscripts in a row differ significantly (P<0.05).

ME available. The comparable partitioning factor also revealed that the efficiency of nutrient utilization from all the licks was similar. These results showed that the incorporation of WB and/ or TP in the licks would not affect nutrient utilization.

4.4 IN *VIVO* EVALUATION OF COMPLETE FEED SUPPLEMENTATED WITH OR WITHOUT DIFFERENT LICKS

4.4.1 Intake of feedstuffs

The daily consumption of different feedstuffs by adult male buffaloes is presented in Table 6 and Fig.1. The daily intake of concentrate mixture was higher (P<0.05) in the control group as compared to those offered UMLs. But within the groups offered UMLs,

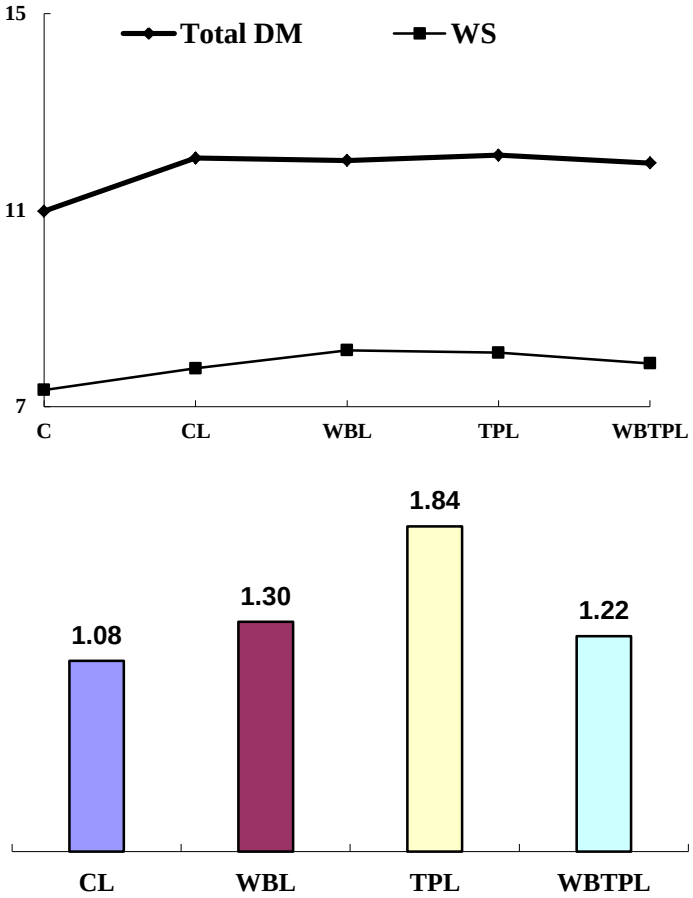
the consumption of the concentrate mixture was comparable. The daily intake of UMLs varied from 1.08 kg (CL) to 1.84 kg (TPL). The consumption of licks containing TP was highest (P<0.05) followed by that containing WB, which was comparable to that containing WBTP. The animals consumed more (P>0.05) wheat straw, when ration was supplemented with UMLs (7.78 to 8.15 kg/d) as compared those offered conventional control diet.

Table 6. Intake of roughage, concentrate and licks, kg/d

Feedstuffs	C	CL	WBL	TPL	WBTPL	PSE
DMI	10.98[a]	12.06[b]	12.01[b]	12.12[b]	11.96[b]	0.12
Concentrate mixture	1.84[b]	0.92[a]	0.92[a]	0.92[a]	0.92[a]	0.09
Lick	--	1.08	1.30	1.84	1.22	0.06
WS	7.34	7.78	8.15	8.10	7.88	0.16
Green	1.01	0.80	0.80	0.80	0.80	0.04
Total roughage	8.34	8.58	8.94	8.90	8.68	0.14
Conc. and lick	1.84	1.99	2.21	2.02	2.13	0.05

Figures with different superscripts in a row differ significantly (P<0.05).

The total intake of roughage (wheat straw and green fodder) and that of concentrate (concentrate mixture and lick) by the animals of different groups was comparable. The higher intake of wheat straw and that of licks in animals offered licks resulted in higher (P<0.05) DM intake, as compared to the one fed control ration. However, the DM intake by the animals offered licks was statistically comparable. The comparable DM intake in all the experimental groups suggested that incorporation of



Licks

Fig. 1 Daily intake of feedstuffs, kg

waste bread and/or tomato pomace did not have any negative effect on palatability of licks, rather improved the consumption of poor quality residues (wheat straw). Tiwari *et al* (1990) and Toppo *et al* (1997) also observed increased consumption of dry matter; in the urea molasses multi nutrient lick supplemented groups. However, Srinivas *et al* (1998) observed a reverse trend.

The concentrate to roughage ratio and green to straw ratio is presented in Table 7. The roughage to concentrate ratio varied from 18:82 to 20:80, while that of green to straw varied from 9:91 to 12:88. The ratios of R: C and that straw to green was maintained within narrow range.

Table 7. The concentrate to roughage ratio in different complete feeds, %

Feedstuffs	C	CL	WBL	TPL	WBTPL	PSE

Concentrate	18.25	18.88	19.82	18.52	19.70	0.40
Roughage	81.75	81.12	80.18	81.48	80.30	0.40
Green	12.44	9.33	8.89	8.94	9.17	0.65
Straw	87.56	90.67	91.11	91.06	90.83	0.64

4.4.2 Intake and digestibility of nutrients

The intake and digestibility of nutrient presented in Table 8 revealed that the DM intake as per cent of the body weight varied from 2.50 to 2.76%, and was high in animals offered UMLs, but the differences were statistically non significant. The water intake (l/d) was also higher (P<0.05) in animals offered UMLs (26.6 to 33.5%) as compared to those offered control ration, which could be due to higher intake of urea and minerals through licks. The water consumption by animals offered licks was statistically comparable. The volume of urine excreted by the animals followed the trend (Fig. 2) of water intake (r = 0.969). The excretion of urine volume from the animals offered UMLs was higher by 57-98% as compared to the animals fed conventional ration.

TABLE 8. SUPPLEMENTATION OF LICKS AND DIGESTIBILITY OF NUTRIENTS

Parameter	C	CL	WBL	TPL	WBTPL	PSE
DMI as % BW	2.50	2.73	2.69	2.76	2.67	0.11
Water, l/d	46.58^{a}	62.10^{b}	62.31^{b}	58.40^{ab}	59.58^{ab}	2.11
Digestibility of nutrients, %						
DM	48.10	52.62	51.64	53.64	51.70	1.43
OM	51.18	55.81	55.89	56.52	55.74	1.25
CP	41.30^{a}	66.83^{b}	65.79^{b}	64.06^{b}	62.77^{b}	2.53
NDF	46.00	47.75	48.25	48.25	47.94	1.44
ADF	38.08	41.44	40.56	41.43	41.08	1.64

Figures with different superscripts in a row differ significantly (P<0.05).

Supplementation of UMLs in the diet of experimental animals improved (P<0.05) the digestibility of crude protein by 52% (WBTP) to 62% (CL) in comparison to un-

supplemented group (C). This was due to higher intake of urea-N, through UMLs, which was rapidly broken down to ammonia and was efficiently utilized by microbes for their proliferation. Tiwari *et al* (1990) and Toppo *et al* (1997) also observed similar results for CP digestibility in UML supplemented groups.

Supplementation of UMLs to the animals improved (P>0.05) the digestibility of DM in comparison to that of control. The improvement in DMD was around 11% when animals were offered TP licks, while the improvement in DMD with CL and with those containing WB (WBL and WBTPL) was 9.5% and 7.5%, respectively. The OMD improved by 9-10% on supplementation of ration with UMLs.

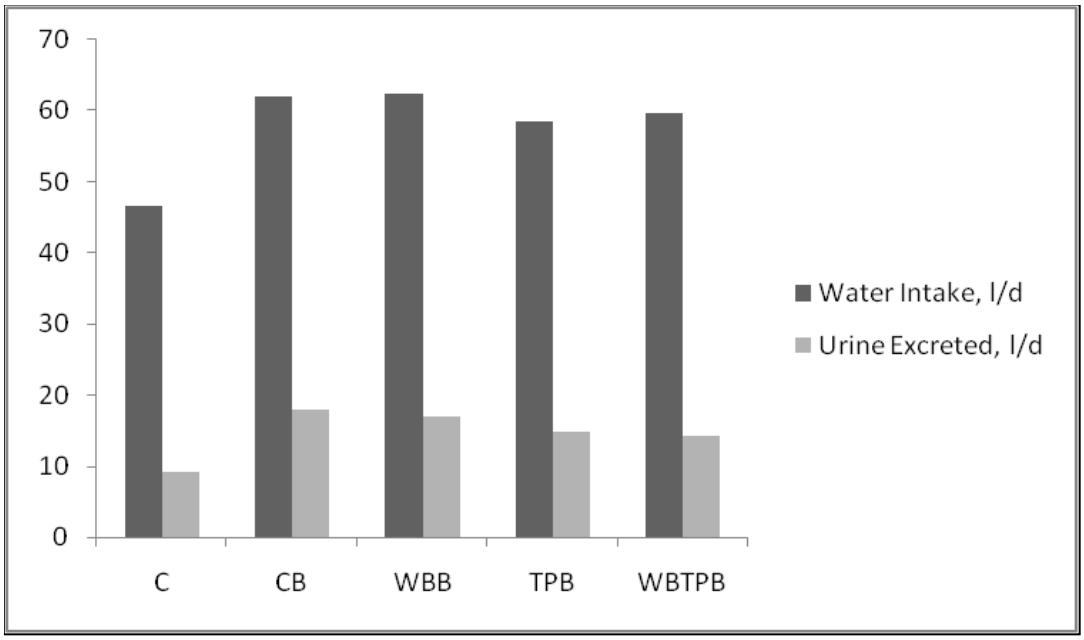


Fig.2. Water intake and urine excretion in animals of different groups

The higher digestibility of different fiber fractions *i.e.* NDF and that of ADF in animals supplemented with UMLs as compared to that in un-supplemented control group, confirming the earlier reports that majority of cellulolytic bacteria proliferate on urea-N resulting into better fiber utilization (Hosamani *et al*, 1998; Tiwari *et al*, 1990, Can *et al*, 2004).

4.4.3 Nitrogen retention and apparent biological value

The N-intake, excretion and its' retention are presented in Table 9. The data showed that there was higher (P<0.05) intake of nitrogen in the animals offered different iso-nitrogenous licks as compared to control group, which could be due to almost double N content in licks than that of conventional control concentrate mixture and higher consumption of lick (1.08 to 1.84 kg as compared to expected intake of 500 g/animal/d). Although there was comparable N excretion through faeces in all groups, but N-excretion through urine was higher (P<0.05) in animals offered UMLs, which was in line with higher intake of water and excretion of urine. The urinary-N excretion was statistically comparable in all the animals offered UMLs.

Table 9. Supplementation of licks and nitrogen retention in adult buffaloes, g/d

Parameter	C	CL	WBL	TPL	WBTPL	PSE
N-Intake	131.57^{a}	201.69^{b}	220.31^{b}	207.14^{b}	213.10^{b}	8.03
Faecal-N	77.00	66.93	74.47	74.77	79.32	2.45
Urinary-N	33.41^{a}	59.71^{b}	53.33^{ab}	56.71^{ab}	51.94^{ab}	3.10
N-outgo	110.41	126.64	127.8	131.48	131.26	5.55
N-Retained	21.16^{a}	75.04^{b}	92.51^{b}	75.57^{b}	81.83^{b}	7.24
BV	15.68	36.88	41.52	36.62	38.44	3.32

Figures with different superscripts in a row differ significantly (P<0.05).

All the animals were in positive nitrogen balance in all the groups. The N retention was higher (P<0.05) in animals offered UMLs as compared to control group. The replacement of wheat with waste bread in the lick improved (P>0.05) the N-retention by 337%. However, replacement of oiled mustard cake (MC) with TP improved the retention of nitrogen by 257%. When both the wheat and MC were replaced with waste bread and

tomato pomace, respectively (WBTPL), the retention of nitrogen was better (287%) than that of conventional lick (255%) and TPL.

The apparent biological value was also higher (134-165%) in the animals supplemented with licks as compared to those in control group. The replacement of wheat with waste bread in the lick improved (P>0.05) the BV by 165%. However, replacement of oiled mustard cake (MC) with TP improved the BV by 134%. An interesting point is that when both the wheat and MC were replaced with waste bread and tomato pomace, respectively (WBTPL), the BV was better (145%) than that of conventional lick (135%) and TPL, clearly indicating that synchronization of available nutrients is important for better utilization.

4.5 IMPACT OF COMPLETE FEED SUPPLEMENTED WITH DIFFERENT UMLS ON RUMEN METABOLITES.

The rumen studies were conducted on three rumen fistulated male buffaloes for assessing the effect of supplementing different licks on the rumen metabolites. The results have been presented in Table 10. The results revealed that TVFA production was comparable in all the groups while pH remained almost constant throughout the study (Table 10), indicating that higher consumption of different licks did not have any adverse effect on rumen environment. Similar trend in pH was observed by Toppo *et al* (2000).

TABLE 10. SUPPLEMENTATION OF UMLS AND RUMEN METABOLITES

Parameter	C	CL	WBL	TPL	WBTPL	PSE
TVFA, meq/dl	9.10	9.47	9.00	9.00	9.13	0.28
pH	6.83	6.85	6.90	6.88	6.80	0.01
			Nitrogen fractions, mg/dl			
Total-N	84.50^{b}	74.97^{ab}	63.50^{a}	66.56^{a}	74.43^{ab}	2.44
TCA-N	48.60	41.83	38.29	42.16	48.32	1.59

NPN-N	35.91[b]	33.13[ab]	23.88[a]	24.40[a]	26.11[ab]	1.60
NH_3-N	11.14	15.46	12.97	12.10	10.39	0.72

Figures with different superscripts in a row differ significantly (P<0.05).

Amongst nitrogen fractions, total nitrogen was higher (P<0.05) in rumen liquor of animals of control group as compared to those offered UMLs. However, the lowest total nitrogen was observed in rumen liquor of animals offered either WBL or TPL. Similar trend (P<0.05) was observed in NPN. Ammonia-N as expected, was non- significantly higher (P<0.05) in rumen liquor of animals offered licks (except the WBTPL), due to intake of N in form of urea through UML, which was rapidly hydrolyzed to form ammonia. The results confirmed the earlier reports (Toppo *et al*, 2000; Jain *et al*, 2005). The efficient utilization of NPN resulted in higher concentration of TCA-N in WBTPL group as compared to other groups. These parameters again confirmed that WBTPL provides nutrients synchronized in energy and protein.

The ruminal flow rate was lower (P<0.05) in animals offered UMLs as compared to control group (Table 11), suggesting higher retention time in the rumen, resulting in better exposure to rumen microbes and in return higher digestibility of nutrients. Rumen volume was higher (P<0.05) in C and WBTPL group while lowest in animals offered CL. The dilution rate was higher (P<0.05) in CL group followed by control group, while lowest in group supplemented with WBTPL.

Table 11. Supplementation of UMLs and rumen outflow rates.

Parameters	C	CL	WBL	TPL	WBTPL	PSE
Rumen volume, l	144.16[b]	73.24[a]	116.46[ab]	120.08[ab]	144.11[b]	8.16
Dilution rate, l/h	0.32[b]	0.38[c]	0.31[ab]	0.30[ab]	0.26[a]	0.01
Outflow rate	45.66[b]	27.83[a]	35.43[ab]	36.16[ab]	37.32[ab]	1.93

Figures with different superscripts in a row differ significantly (P<0.05).

4.5 EFFECT OF THE COMPLETE FEED SUPPLEMENTED WITH DIFFERENT UML ON BLOOD CONSTITUENT

The effect of UML supplemented diet on blood biochemical constituents have been presented in Table 12. The results revealed that blood glucose level (indicator of energy status) varied from 48 mg/dl (C) to 58.5mg/dl (TPL); the values were within the normal limit reported by Boyd (1984).

Table 12. Supplementation of UMLs and blood profile, mg/dl

Parameter	C	CL	WBL	TPL	WBTPL	PSE
Glucose	48.14	49.75	47.52	58.52	50.55	1.74
Urea	21.32[a]	47.48[b]	44.28[b]	38.25[b]	44.20[b]	2.54
Creatinine	1.91	2.25	1.82	1.96	2.00	0.09
Total protein, g/dl	6.64	7.30	7.66	7.54	6.76	0.25
Albumin(A), g/dl	1.90	2.08	2.30	2.16	2.12	0.07
Globulin(G), g/dl	4.75	5.22	5.36	5.38	4.56	0.21
A:G	0.40	0.40	0.45	0.41	0.51	0.02
Cholesterol	63.04[b]	60.10[ab]	48.39[a]	57.39[ab]	46.13[a]	2.01
Triglycerides	25.4	29.8	30.8	24.1	23.9	1.58
Ca	10.45	10.82	11.62	11.59	10.96	0.32
P	8.18	9.31	10.22	11.36	11.06	0.66

Figures with different superscripts in a row differ significantly (P<0.05).

The values of the total protein in blood plasma of the animals were found to be within the range of reported values (Jain, 1996). There was low (P>0.05) plasma globulin concentration (4.56g/dl) in WBTPL fed group with an increase in albumin/globulin ratio (0.51) than that the value of C, CL or TPL fed groups. The mean value of plasma albumin

was low in animals fed conventional ration, which showed a little lower from the normal range recommended by Boyd (1984) as compared to those fed UMLs.

The blood urea (BU) was comparable between the animals offered diet supplemented with different licks, but higher ($P<0.05$) than that of control group, which could be due to higher intake of nitrogen through UML. Although, high BU in treatment groups no symptoms of urea toxicity was observed during the study period and all animals were found to be active and in good health. Urea-N normally increases with the increasing intake of protein and non-protein nitrogen (Huber *et al*., 1976). The blood urea-N level was almost double (21 v/s 47 mg/dl) in animals offered UMLs supplemented diet as compared to conventional ration, an indicator of excessive release of ammonia in rumen, which could not be efficiently utilized by the rumen microorganisms. The possibility of less heat generation (by CaO during mixing) required to produce maillard product, could not be ruled out.

In the animals fed UMLs, the serum creatinine (CRT) was higher than animals fed conventional ration, however amongst animals offered UMLs, the animals offered licks containing WB showed lower values and those fed on CL showed higher values of CRT. Supplementing of UMLs in the diet showed reduction ($P<0.05$) in mean concentration of serum cholesterol with lowest value for group supplemented with WBTPL. The level of cholesterol was lower than the normal range (Kaneko, 1997). The addition of 15% of tomato pomace to the diet reduced serum cholesterol level by nearly 50% (Bobek, 1998).The levels of triglycerides varied from 23.9 (WBTPL) to 30.8 (WBL).

The plasma inorganic phosphorus concentration in Table 12, it was higher ($P>0.05$) in animals fed UMLs than that of animals fed conventional ration. However, both the mean values were within the range of values reported by Singh *et al.* (1990). The values of serum Ca varied from 10.5 to 11.6mg/dl, and were within the physiological range (Kaneko, 1997).

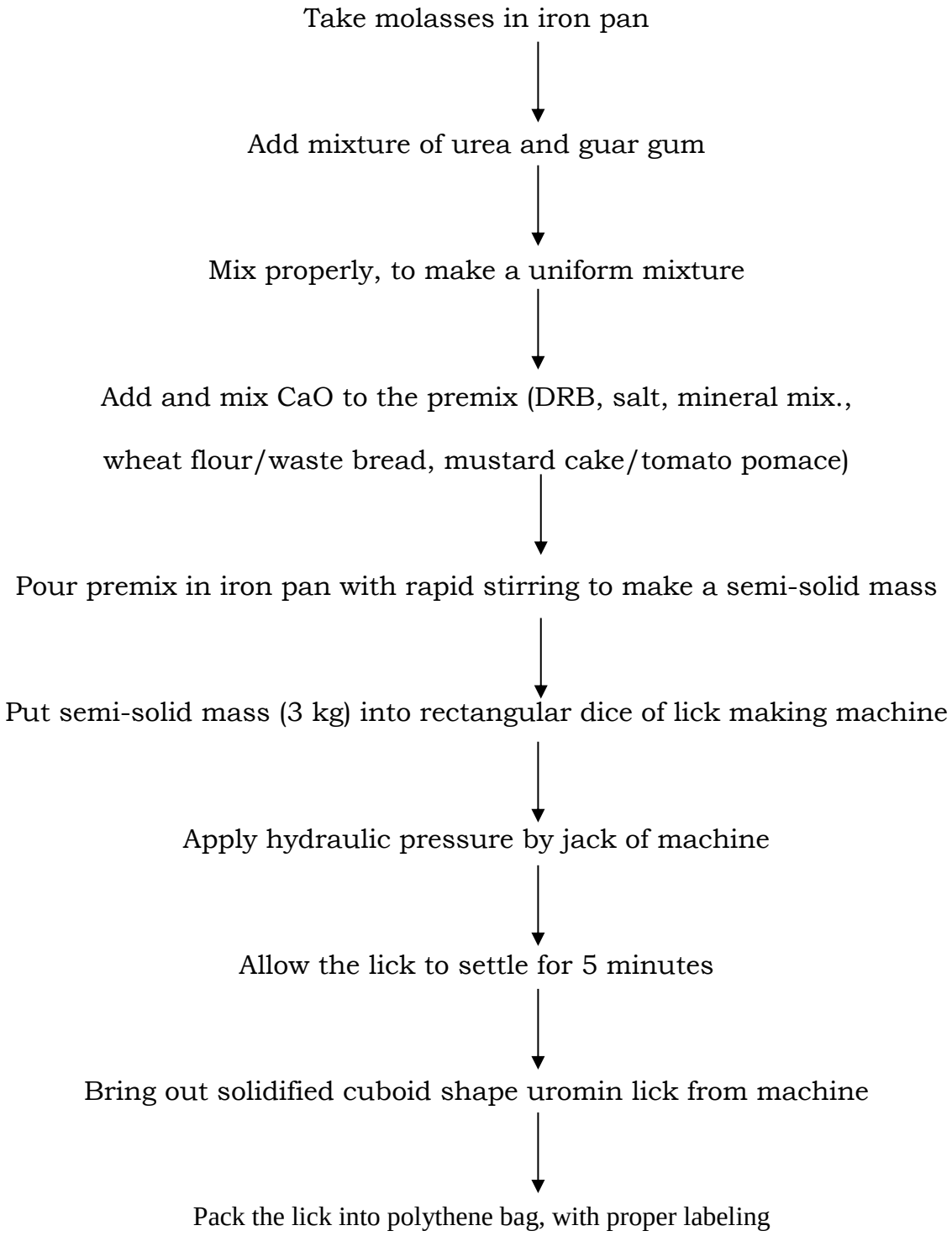
4.6 CHANGES IN BODY WEIGHT

The effect of different diet on the weight changes has been presented in Table 13. The change in body weight in all the animals was in positive direction *i.e.* gained weight. The animals supplemented with TPL have shown highest gain/d (603.85 g/d) while gain was lowest (350.18 g/d) for un-supplemented control group. The gain in weight of all the animals offered lick was higher than that of control group, but, the differences were statistically non significant.

Table 13. Supplementation of UMLs and performance of animals

Parameter	C	CL	WBL	TPL	WBTPL	PSE
Initial BW, kg	437.98	441.05	445.58	438.28	447.85	6.39
Final BW, kg	450.64	451.65	457.50	454.25	466.52	4.64
Gain in BW g/d	350.18	386.74	409.72	603.85	359.55	107.58

The results discussed conclusively revealed that UML has an edge over conventional concentrate mixture and non conventional ingredients like waste bread and tomato pomace could be incorporated into UMLs depending on availability without any adverse effect on palatability, nutrient utilization or health of animals. Above all the preparation of UML could be economized and conventional ingredients could be spared for more vulnerable species.



Flow diagram showing preparations of uromin lick (UML)

CHAPTER-V

SUMMARY

The present study was undertaken to formulate and compare uromin lick (UML) containing alternate energy and protein supplements like waste bread (WB) and tomato pomace (TP) with conventional block and to assess the effect of such UMLs on nutrient utilization in buffaloes. Waste bread was procured from Cremica Industries, Phillaur. The waste bread was sun dried for 14 hours and got tested for the level of mycotoxins, from the Department of Veterinary Microbiology, GADVASU. The sun dried waste bread had negligible level of mycotoxins. The tomato pomace (containing tomato peels, seeds and damaged tomatoes) was procured from Nijjar Agro Industries, Amritsar. The tomato pomace was sun dried and finely ground.

Iso-nitrogenous and iso-caloric licks were prepared by manipulation of feed ingredients. The wheat flour from conventional UML was replaced with waste bread and oiled mustard cake with tomato pomace. The required quantity of molasses and urea were weighed and mixed in a 25 kg capacity iron pan. The guar gum was added to the urea-molasses mixture as binder. A premix of other ingredients was prepared (CaO was the last ingredient added to this premix) and added to iron pan with rapid stirring. Two UMLs were prepared at a time in a manually operated block-making machine. These licks were analyzed for proximate constituents and different cell wall fractions. The digestibility of nutrients and availability of ME from different UMLs was assessed by *in vitro* gas production technique. The data of chemical composition, *in vitro* evaluation and *in vivo* studies were evaluated by simple ANOVA by using SPSS ver. 12 and means were compared by using Tukey b.

The waste bread contained 12.5% CP and an excellent source of cooked starch. The dried tomato pomace proved to be an excellent source of both proteins (20.9%) and fat

(11.0%). The cell wall constituent i.e. NDF, ADF, cellulose and hemicellulose were much higher in tomato pomace as compared to that in waste bread.

The chemical analysis of different UMLs showed that total ash varied between 26.5 to 27.6% and was comparable amongst all UMLs. The high ash content in the licks could be due to high level of mineral mixture used. The CP and EE content of different UMLs was similar indicating that licks were isonitrogenous and isocaloric. The cell wall constituents in the conventional block (CL) and block containing waste bread (WBL) were lower than the licks containing tomato pomace (TPL and WBTPL), mainly because of higher fiber fraction in tomato pomace.

The net gas production and digestibility of nutrients (OM and NDF) was similar in all the licks. The *in vitro* release of ammonia during fermentation was also statistically comparable. The total volatile fatty acid production varied from 8.95 meq/dl (TPL) to 9.75 meq/dl (CL). Replacement of cereal grains with WB (WBL) showed no significant effect on the production of TVFA, and level was statistically comparable ($P>0.05$) to that of CL, However, replacement of mustard cake with TP alone (TPL) resulted in significant depression in TVFAs concentration as compared to CL. A combination of WB and TP (WBTP) proved to be a better option as far as production of VFAs and availability of ME was concerned. The availability of ME (MJ/kg DM) from different licks varied from 5.71 (WBL) to 6.03 (WBTPL). The comparable Partitioning factor also revealed that the efficiency of nutrient utilization from all the UMLs was similar. The results showed that the incorporation of WB and/ or TP in the licks would not affect nutrient utilization.

In order to assess the effect of different UMLs on actual nutrient utilization in animal system, adult male Murrah buffaloes (20; 5-6 year old of 442.1±6.3 kg BW) randomly distributed into five equal groups were offered either 2 kg conventional concentrate mixture (maize 30, mustard cake 10, solvent extracted mustard cake 20, rice bran 15, solvent extracted rice bran 22, mineral mixture 2 and common salt 1 per cent each)

while in the experimental groups, the animals were offered 1 kg concentrate mixture and *ad lib* respective UML supplemented with 5 kg green fodder and 9 kg wheat straw. At the termination of 30 day experimental feeding, a 7-day metabolic trial was conducted on all the animals.

The daily intake of concentrate mixture was higher ($P<0.05$) in the control group as compared to those offered UMLs, but statistically comparable with in licks supplemented groups. The daily intake of block varied from 1.08 kg (CL) to 1.84 kg (TPL). The consumption of licks containing TP was highest followed by that containing WB (WBL &/or WBTPL). The animals consumed more ($P>0.05$) wheat straw, when ration was supplemented with UMLs (7.78 to 8.15 kg/d) as compared those offered conventional control ration. The higher intake of wheat straw and that of licks in animals offered licks resulted in higher ($P<0.05$) DM intake, as compared to the one fed control ration. However, the DM intake by the animals offered licks was statistically comparable, indicating that waste bread and/or tomato pomace did not have any negative effect on palatability of licks. The roughage to concentrate ratio varied from 18:82 to 20:80, while that of green to straw varied from 9:91 to 12:88. The values were comparable in all the groups. The DM intake as percent of the body weight varied from 2.50 to 2.76%, but the differences were statistically non significant. The water intake (l/d) was higher ($P<0.05$) in animals offered UMLs (26.6 to 33.5%) as compared to those of control ration, which could be due to higher intake of urea and minerals through licks. The volume of urine excreted by the animals follow the trend of water intake ($r = 0.969$).

Supplementation of UMLs in the diet of experimental animals improved ($P<0.05$) the digestibility of crude protein by 52% (WBTP) to 62% (CL) in comparison to un-supplemented group (C). The digestibility of DM, OM, NDF and that of ADF in animals supplemented with UMLs was comparable to that in un-supplemented control group.

The data showed that there was higher (P<0.05) intake of nitrogen in the animals offered different iso-nitrogenous licks as compared to control group, which could be due to two reasons, one because the consumption of block was more than the double *i.e.* 1.08 to 1.84 kg as compared to expected intake of 500 gm/animal/day; secondly almost double N content in licks as compared to the conventional control concentrate mixture. Although there was comparable N excretion through faeces in all groups, but N-excretion through urine was higher (P<0.05) in animals offered UMLs. The urinary-N excretion was statistically comparable in all the animals offered UMLs. All the animals were in positive nitrogen balance in all the groups. The N retention was higher (P<0.05) in animals offered UMLs as compared to control group. When both the wheat and MC were replaced with waste bread and tomato pomace, respectively (WBTPL), the retention of nitrogen was better than that of conventional block and TPL.

Simultaneously, rumen studies were conducted on three rumen fistulated male buffaloes for assessing the effect of supplementing different licks on the rumen metabolites. The results revealed that TVFA production was comparable in all the groups while pH remained almost constant throughout the study, indicating that higher consumption of different licks did not have any adverse effect on rumen environment. Amongst nitrogen fractions, total nitrogen and NPN were higher (P<0.05) in rumen liquor of animals of control group as compared to those offered UMLs. Ammonia-N as expected, was non- significantly higher (P>0.05) in rumen liquor of animals offered licks. The efficient utilization of NPN resulted in higher (P<0.05) concentration of TCA-N in WBTPL group as compared to other groups. These parameters again confirmed that WBTPL provides nutrients synchronized in energy and protein.

The ruminal flow rate was lower (P<0.05) in animals offered UMLs as compared to control group, suggesting higher retention time in the rumen, resulting in better exposure to rumen microbes and in return higher digestibility of nutrients. Rumen volume was higher (P<0.05) in C and WBTPL group while lowest in animals offered CL. The dilution

rate was higher ($P<0.05$) in CL group followed by control group, while lowest in group supplemented with WBTPL.

The blood profile of the animals in all the groups was comparable except the blood urea nitrogen (BUN), which was comparable between the animals offered diet supplemented with different licks, but was higher ($P<0.05$) than that of control group. It could be due to higher intake of nitrogen through UML. Although, BUN was high in treatment groups, no symptoms of urea toxicity was observed during the study and all animals were active and in good health. All the blood parameters were with in the normal physiological range. The gain in weight of all the animals offered block was higher than that of control group, but, the differences were statistically non significant.

It was concluded that non-conventional ingredients like waste bread and tomato pomace could be incorporated into UMLs without any adverse effect on palatability, nutrient utilization, rumen metabolites or health of animals. Above all the preparation of UML could be economized and conventional ingredients could be spared for more vulnerable species.

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