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ORIGINAL ARTICLE

Nutritional and Keeping Quality of Total Mixed Ration Briquettes Produced for Lactating Dairy Cows

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Abstract

This research aims to find out the feasibility of the formation of different total mixed ration (TMR) briquettes. Six recipes (TMR1, TMR2, TMR3, TMR4, TMR5, and TMR6) were prepared by blending different combinations of forages and concentrates to balance the nutrient requirements of lactating dairy cows. Each TMR briquette was wrapped and stored at room temperature for three months and tested at monthly intervals for nutrient composition and shelf life. A cost analysis was performed to determine economic feasibility. The crude protein, ether extract, crude fibre, acid detergent fibre, neutral detergent fibre, nitrogen-free extract and non-fibre carbohydrate contents were not significantly different among the treatments at preparation and after 3 months of storage. Calcium, phosphorous and magnesium contents of the treatments were significantly different among the treatments at preparation and after three months of storage. There was no risk associated with the potentially toxic metals (Cd, Pb and Hg) in the treatments during the storage. Yeast & mould counts, total plate counts and free fatty acid contents were in the acceptable range in all treatments during the entire storage period. Weights of the briquettes were significantly decreased during the storage; however, heights were not different among the treatments. TMR5 and TMR6 resulted lower production costs per kg. The majority of the nutritional, shelf life and physical parameters were not significantly different among the six TMR recipes tested. Thus, 5 and 6 TMR briquettes were selected as the best two potential recipes for further studies.

Keywords: *Nutrient composition, Physical parameters, Production cost, Shelf life*

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1. Introduction

In the past, dairy cows were fed mainly with a forage-based diet supplemented with concentrates to provide some extra nutrients. In temperate countries, cows are provided with hay or silage during the cooler seasons as the main diet (Schingoethe 2017) and use pasture based-diets during the spring as the cost of production is less (Schären et al. 2016). However, forage-based diets are not enough to meet the daily nutritional demand of high producing cows, thus the importance of feeding mixed rations included with different feed ingredients has emerged.

Total Mixed Rations (TMR) are a blend of forages and industrial by-products such as cereal by-products, distillery by-products, cereal hulls and oilseed meals with feed additives (Coppock et al. 1981). It can be formulated to different animal groups i.e. growing animals, dairy cattle or beef cattle incorporating a variety of feed ingredients locally available (Lammers et al. 2003). The special feature of TMR is that every bite supplies an adequate amount of nutrients improving feeding efficiency (Premaratne and Samarasinghe 2020). Research has shown that the imbalance in crude protein and energy levels in pasture-based diets supplemented with concentrates could be avoided by feeding nutritionally balanced TMR (Kolver and Muller 1998; Bargo et al. 2002).

At present, feeding TMR prepared according to different recipes has become popular in Sri Lanka (Weerasinghe 2019). However, as the availability of feed ingredients is not consistent throughout the year, the composition of TMR is also varied. As a result, the milk production and the Body Condition Score (BCS) of the cows fluctuate depending on the feed quality (Sova et al. 2013; Roche et al. 2006). Unavailability of quality feed resources in the required quantity is one of the major problems faced by the dairy farmers in the dry zone of Sri Lanka, especially during the dry periods (January to March and July to September) (Houwers et al. 2015). The majority of the farmers pay less attention on feeding cattle with a nutritionally balanced diet due to lack of knowledge and attention. Similarly, most of the farmers practise an extensive or semi-intensive system of management in the dry zone (Vidanaarachchi et al. 2019). Thus, the farmers mainly depend on the forages available near roadsides, paddy field bunds, tank valleys, marginal lands and *villues* (Premaratne and Somasiri 2015; Premaratne and Samarasinghe 2020). The fluctuation in the quantity and quality of forage in the above feeding grounds results in inconsistent milk production and a sudden drop of BCS in cows. Therefore, TMR feeding can be a better remedy for such situations to maintain consistent milk production and BCS.

Research has shown that leaf-meals can be preserved as briquettes/blocks to reduce the bulkiness, increase keeping quality, and facilitate easy transport and storage (Somasiri et al*.* 2010). Premaratne and Samarasinghe (2020) stated that leaf-meal blocks mixed with other concentrate feed ingredients provide a quality feed for ruminant livestock improving the feed digestibility. Thus in order to supply a consistent quality and a balanced diet throughout the year for dairy cattle especially in dry zone areas formulated TMR could be introduced. These formulated TMR could be stored in a form of a briquette. It helps to overcome under-feeding practices, reduce feed wastage, and save labour cost and time. Further, it improves the productivity of dairy cows (FAO 2012). Hence, the livestock farmers may enjoy a stable income throughout the year by feeding these TMR briquettes.

Previous studies have shown that the majority of livestock farmers in Sri Lanka undertake farming as a secondary source of income (Perera and Jayasuriya 2008). Hence, if a TMR briquette is available in the market, farmers could purchase the TMR briquette and avoid the difficulties in finding forages or concentrates daily. Therefore, the development of a commercially viable TMR briquette would be a better solution to overcome the difficulties faced by dairy farmers especially in the dry zone of Sri Lanka. Thus, this research aimed to produce different TMR recipes using locally available feed ingredients, find out the feasibility of these recipes forming into briquettes and assess the keeping quality. This enables the selection of the two best TMR recipe briquettes considering shelf life (keeping quality), physical properties, nutritive composition, and cost analysis.

2. Materials and Methods

Experimental Site

This study was conducted at the Faculty of Agriculture, Rajarata University of Sri Lanka (8.2749° N, 80.6362° E). The forage species were collected from the surrounding area closer to the faculty. The concentrate ingredients were purchased from the dealers in *Anuradhapura* city, Sri Lanka. Molasses was obtained from *Gal-Oya* Plantations (Pvt) Limited, *Hingurana*. The mineral mixture and di-calcium phosphate were purchased from the local market. The experiment received the approval from the Animal Ethics Committee, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Sri Lanka (VERC-19-09).

Preparation of Different TMR Recipes

Different locally available grasses; Hybrid Napier – CO-3 (*Pennisetum perpureum X Pennisetum americarnum*), Guinea grass species (*Panicum maximum*), Maize (*Zea mays*), Sorghum (*Sorghum bicolour)* and Gliricidia (*Gliricidia sepium*) were collected. The guinea grass and CO-3 were harvested at the pre-blooming stage. The entire plant of maize was harvested before cob initiation, and sorghum was harvested 60 days after planting. The leaves and twigs from mature Gliricidia trees were harvested. These samples were chopped into particles below 2.5 cm and air-dried under shade to reduce moisture level up to 15 – 25%. The air-dried forage samples were stored in a dry place until further use. Concentrate ingredients; coconut (*Cocos nucifera*) poonac, maize meal, rice (*Oriza sativa*) bran and Soybean (*Glycine max*) Meal (SBM) were ground and stored in a dry place until further use. Molasses was used in liquid form without boiling.

Six TMR recipes (treatments in this experiment) were prepared by blending above stated locally available ingredients in different combinations according to the nutrient requirements for lactating dairy cows (4.5% milk fat, 10 litres of milk yield per day) following the recommendations (NE_L=15.7 Mcal, CP=11.8%, Ca=0.43%, P =0.28%) given by NRC (2001). The treatments were arranged according to a Completely Randomized Design (CRD) including four replicates. Each TMR recipe was prepared and ingredients were mixed properly by hand and using a mechanical mixture (Vmamix, Vietnam) according to Table 1. Each recipe mixture was then pressed into briquettes of 1 kg by applying hydraulic pressure using a briquette machine (Green Pack 09, Sri Lanka). Each briquette was wrapped with polythene (gauge 300) and sealed using a tape. The recipe number, date, height and weight were recorded and all the briquettes were stored at room temperature for three months. Thus, altogether there were 72 briquettes (6 treatments x 4 replicates x 3 months). Randomly selected four briquettes (replicates) from each treatment were used for the analysis of storage quality at monthly intervals.

Sample Analysis and Data Collection

The stored briquettes were observed for changes in colour, weight and height during storage in monthly intervals.

Table 1. Composition of total mixed ration recipes prepared by mixing different forage and concentrate ingredients

Treatment (recipe)[1] / Ingredient (%)	T1	T2	T3	T4	T5	T6
Gliricidia	6.5	5	10	11	9	11
Guinea grass	23	17	18.5	0	21.5	14
Maize	0	24	27	11.5	16	10.5
CO-3	27	26	0	32	13	19
Sorghum	14	0	21	14	13	22
Rice bran	14	10	0	1	9.5	6.5
Maize meal	8.5	0	11.5	7.5	2.5	7
Soybean meal	5	5	6	0	2.5	3
Coconut poonac	0	5	4	14	11	5
Molasses	0	6	0	7	0	0
Mineral mixture[2]	2	0	2	0	2	0
Di-calcium phosphate	0	2	0	2	0	2
	100	100	100	100	100	100

1T1 to T6 different TMR recipes
2 Super Feed Pvt. Ltd., Sri Lanka.

The colour was measured and the values of lightness (L*), redness (a*), and yellowness (b*) were obtained at six sites on the respective TMR briquette using Colourmeter (Konica Minolta, CR 10, Japan). The weight and height of the briquettes were obtained using a weighing balance (WEIGHTECHROLEX 2P-15B, China) and a standard metric ruler. Then, each TMR briquette was opened and mixed thoroughly. A sub-sample was taken from each briquette for the determination of shelf life and nutritional composition.

Nutrient composition was analysed using proximate analysis procedures given by AOAC, (2019); Dry Matter (DM) by the hot air oven at 105 ^{o}C (YCO-010, Taiwan), Crude Protein (CP) by Kjeldahl method (DK 20, Italy), Crude Fibre (CF) by using fibre analyzer (FIWE3, Italy), Ether Extract (EE) by using soxhlet extractor (MICROSIL, India) and ash by using muffle furnace (DMF-05, Korea). Acid Detergent Fibre (ADF) and Neutral Detergent Fibre (NDF) were analysed according to Van Soest (1991) on Dry Matter (DM) basis. Gross energy content was analysed using a bomb calorimeter (C200 Auto, Germany). Phosphorus (P) content was analysed using an atomic absorption spectrophotometer (LABOMED, USA). Total calcium (Ca), magnesium (Mg) and potentially toxic metals (Cd, Pb, As & Hg) were analysed using an inductive coupled plasma optical emission spectrophotometer (Icpap7400 Duo MFC, USA) and following the procedure given by Chapman and Pratt (1961) with slight modifications. Where 0.5 g of sample was ashed for 5 hrs at 550 °C and ash was dissolved in 5 mL of 2N HCl.

Data related to shelf life were collected by analysing each briquette for Free Fatty Acid content (FFA), Total Plate Count (TPC) and, Yeast and Mould Count (Y&MC). The FFA content in the sample was determined as described by Pearson (1973). Microbial contamination was decided for each briquette by obtaining TPC and Y&MC according to Goeser (2016). Plate count agar (Biolab Zrt, Hungary) and potato dextrose agar (Biolab Zrt, Hungary) were used as the growing media for TPC and Y&MC, respectively. All the inoculated plates were incubated at 37°C and the enumerated plates were kept at room temperature for 24 hrs and 72 hrs, respectively. The total number of colonies were counted using a colony counter (Galaxy 330, Taiwan) to obtain TPC and Y&MC for each briquette.

Nitrogen Free Extract (NFE), Non-Fibre Carbohydrate (NFC), Metabolizable Energy (ME) and Total Digestible Nutrients (TDN) values were calculated using Equations 1, 2, 3, 4, 5 and 6, respectively.

$$NFE\ (\%) = 100 - (Ash\ (\%) + CF(\%) + EE(\%) + CP\ (\%)$$ (Kearl 1982) (Eq. 1)

$$NFC\ (\%) = 100 - (NDF(\%) + CP(\%) + EE(\%) + Ash(\%)$$ (Weiss and Tebbe 2019) (Eq. 2)

$$ME\ (Mcal/kg) = \left(\left(1.01x(TDN\%\ x\ 0.04409)\right) - 0.45\right)$$ (NRC 2001) (Eq. 3)

$$TDN\ (\%)\ (Dry\ forrage\ and\ roughages) = -17.26 + 1.212\ (CP) + 0.8352\ (NFE) + 2.464\ (EE) + 0.4475\ (CF)$$ (Kearl 1982) (Eq. 4)

$$TDN\ (\%)(Eergy\ feeds) = 40.26 + 0.1969\ (CP) + 0.4228\ (NFE) + 1.190(EE) - 0.1379(CF)$$ (Kearl 1982) (Eq. 5)

$$TDN\ (\%)\ (Protein\ supplements) = 40.32 + 0.5398\ (CP) + 0.4448\ (NFE) + 1.422\ (EE) - 0.7007\ (CF)$$ (Kearl 1982) (Eq. 6)

Where, NFE - Nitrogen Free Extract, CF - Crude Fibre, EE - Ether Extract, CP - Crude Protein, ME - Metabolizable Energy and TDN - Total Digestible Nutrients.

Cost Analysis for Each Recipe

The cost of production of each recipe was calculated using the expenditure related to purchasing ingredients, labour cost involved for drying and preparation of TMR briquettes, cost of packaging materials and electricity.

Selection of Best Two Recipes

The best two recipes were selected based on; physical properties, nutrient composition, shelf life, and cost of production.

Data Analysis

Effects of treatment and period on physical properties (colour, weight and height), nutrient composition (DM, CP, EE, CF, NDF, ADF, NFE, NFC, TDN, GE, ME, ash, Ca, P, Mg), potentially toxic metals (Cd, Pb, As & Hg) and shelf life (FFA, TPC and Y&MC) were assessed using an ANOVA. Means were separated using Tukey's Studentized Range Test (TSRT) and statistical significance was declared at $p < 0.05$.

3. Results and Discussion

Physical Properties of TMR Briquettes

The differences in colour (lightness (L*), redness (a*) and yellowness (b*)) have not significantly changed within a period except for redness (a*) in the third month of storage (Table 2).

There was no significant height reduction in the TMR recipe briquettes during the storage period in the present study. However, there was an increasing trend of weight loss during storage. This may associate with the loss of moisture from the TMR ingredients. In contrast, Somasiri et al. (2010) found that during the three months of storage there was no significant weight loss in the feed blocks prepared using leguminous leaf meals.

Nutrient Profile of TMR Briquettes

Nutrient profiles for six treatments (TMR recipe briquettes) are presented in Table 3. The nutritional composition of individual ingredients was evaluated and already published (Karunanayaka et al. 2020a, b, c).

Table 2. Physical properties of total mixed ration briquettes in three months storage

Month	Treatments						SE[1]	*p* value[1]
	1	2	3	4	5	6		
	Lightness (L*)							
Initial	37.92^{B}	39.88	39.37^{B}	38.92^{B}	38.61^{B}	39.04^{B}	0.66	0.436
1	44.81^{A}	43.72	42.90^{AB}	43.72^{AB}	43.12^{AB}	43.88^{AB}	1.69	0.975
2	42.40^{AB}	43.72	47.02^{A}	46.13^{A}	45.38^{A}	45.09^{A}	1.62	0.414
3	44.99^{A}	46.33	44.46^{AB}	45.60^{AB}	45.51^{A}	42.52^{AB}	2.11	0.847
SE[2]	1.51	2.16	1.63	1.64	1.38	1.18		
p value[2]	0.021	0.261	0.039	0.035	0.014	0.019		
	Redness (a*)							
Initial	3.10	3.31	3.01^{AB}	3.21^{B}	3.26	3.24	0.09	0.229
1	3.07	4.16	2.34^{B}	3.63^{AB}	3.06	3.40	0.59	0.399
2	3.46	3.42	3.61^{A}	4.19^{AB}	3.08	3.58	0.34	0.364
3	3.47^{ab}	2.77^{ab}	3.59^{Aab}	4.45^{Aa}	2.66^{b}	3.54^{ab}	0.39	0.050
SE[2]	0.41	0.47	0.29	0.26	0.51	0.37		
p value[2]	0.830	0.282	0.028	0.023	0.862	0.911		
	Yellowness (b*)							
Initial	17.84	19.46	18.91	18.37	19.18	19.11	0.50	0.251
1	19.30	19.57	18.44	17.92	17.54	17.52	0.57	0.080
2	18.67	18.36	19.36	19.29	18.31	18.07	0.64	0.628
3	19.00	21.04	17.56	19.20	19.11	17.57	0.87	0.101
SE[2]	0.79	0.78	0.74	0.56	0.60	0.39		
p value[2]	0.609	0.168	0.396	0.296	0.232	0.045		
	Height reduction, %							
Initial	0	0	0	0	0	0	-	-
1	1.71	2.34	4.79	1.62	1.42	0.94	2.47	0.901
2	9.01	6.06	7.74	7.06	5.83	8.47	3.83	0.988
3	5.43	7.54	2.28	8.09	10.74	7.47	4.17	0.798
SE[2]	3.74	4.14	3.83	3.23	3.16	3.19		
p value[2]	0.421	0.669	0.618	0.357	0.170	0.246		
	Weight loss, %							
Initial	0	0	0	0	0	0	-	-
1	0.23^{Ba}	0.19^{Ca}	0.18^{Ba}	0.74^{Bc}	0.41^{b}	0.59^{b}	0.12	0.021
2	1.27^{Ab}	2.01^{Bab}	1.64^{Aab}	3.75^{Aa}	1.97^{ab}	1.73^{ab}	0.49	0.032
3	1.47^{Ab}	4.51^{Aa}	1.93^{Aab}	3.02^{Aab}	2.30^{ab}	2.85^{ab}	0.58	0.027
SE[2]	0.26	0.37	0.24	0.31	0.58	0.71		
p value[2]	0.018	<0.001	0.001	0.0002	0.096	0.138		

a, b, c mean values were significantly ($p < 0{\cdot}05$) different within the row across treatments
A, B, C mean values were significantly ($p < 0{\cdot}05$) different within the column across the storage period
[1] Standard error (SE) and P-value across the treatments
[2]SE and *p*-value across the storage periods

Dry matter (DM) content

The DM content was significantly different among the treatments during different storage periods (Table 3). Dry matter content was significantly increased in all the treatments with storage time. It may be due to further reduction of moisture content in the ingredients in the TMR as a result of evaporation. The briquettes were not 100% vacuum packed, but covered with polythene and tightly sealed.

Lahr et al. (1983) stated that if the DM content is less than 45%, it reduces the Dry Matter Intake (DMI) in cows due to gut fill as the feed contains a higher amount of moisture. In the present study, TMR rations were prepared using dried ingredients. Thus, the DM% was above 87% in all the TMR recipe briquettes. It can be expected that high DM content may have a negative effect on feed intake. Therefore, it is necessary to moisturize the TMR briquettes before feeding, which is a completely different procedure compared to other TMR already available in the market. We believe that it will not negatively affect the DMI of the cows.

Crude Protein (CP) content

After 3 months of storage, the CP contents were similar among the treatments. However, the CP content of T3 and T4 was significantly higher at three months of storage compared to the initial CP. The exact answer for this increase is not known but we can assume that the microbial proteins may have also contributed to the increase in CP content. As shown in Table 6, microbial growths were increased across periods within all treatments.

Crude protein content in the present TMR recipe briquettes was varied between 10 to 12%. Kulathunga et al. (2015) observed a CP content of 9.0 – 14.5% in feed blocks prepared with different agricultural waste. Dietary CP levels is an important factor that affects different production responses (Hristov et al. 2004). Increased CP levels increased the milk production of the cow however, the excess is considered undesirable. Hence, the excess is excreted leading to environmental pollution and profit loss (Cherdthong et al. 2011). Therefore, it is important to feed the required amount of CP avoiding excess.

Ether Extract (EE) content

The EE contents were not significantly different among the treatments when considered the different periods (Table 3). The EE content was drastically reduced by the third month of the storage period compared to the initial EE content in all treatments. Since, EE composed of heterogeneous materials such as galactolipids, triglycerides and phospholipids and all other non-polar compounds such as phosphatides, steroids, pigments, fat-soluble vitamins, and waxes; some compound may be volatile or remove with time.

Table 3. Proximate composition of total mixed ration briquettes in three months storage

Month	Treatments						SE[1]	*p* value[1]
	1	2	3	4	5	6		
	Dry Matter (DM), %							
Initial	89.6^{Cab}	87.2^{Cc}	89.9^{Ca}	87.9^{Bbc}	89.3^{Bab}	89.5^{Bab}	0.4	0.001
1	91.2^{Ba}	89.9^{Bb}	90.6^{BCab}	87.4^{Bc}	90.8^{Aab}	90.7^{Aab}	0.3	<0.001
2	93.5^{Aa}	91.1^{Abc}	92.3^{ABab}	90.0^{Ac}	91.4^{Abc}	90.9^{Abc}	0.3	<0.001
3	93.2^{Aa}	91.4^{Abcd}	92.6^{Aab}	90.3^{Ad}	91.8^{Abc}	91.2^{Acd}	0.3	<0.001
SE[2]	0.2	0.3	0.4	0.5	0.3	0.3		
p value[2]	<0.001	<0.001	0.001	0.001	<0.001	0.003		
	Crude Protein (CP), %							
Initial	10.0^{B}	10.1^{C}	10.2^{B}	10.0^{B}	10.1^{B}	10.3^{A}	0.2	0.935
1	10.4^{Bc}	11.6^{ABab}	11.9^{Aa}	11.0^{ABbc}	11.6^{Aab}	11.1^{Abc}	0.2	<0.001
2	11.6^{Aab}	11.9^{Aab}	11.9^{Aab}	11.1^{ABb}	12.1^{Aa}	11.2^{Aab}	0.2	0.008
3	10.5^{B}	10.7^{BC}	11.6^{A}	11.2^{A}	10.8^{B}	10.8^{A}	0.3	0.255
SE[2]	0.2	0.3	0.2	0.3	0.2	0.3		
p value[2]	0.001	0.001	<0.001	0.040	<0.001	0.285		
	Ether Extract (EE), %							
Initial	3.6^{A}	3.4^{A}	4.0^{A}	3.2^{A}	4.1^{A}	3.5^{A}	0.3	0.406
1	2.0^{B}	1.9^{B}	1.9^{B}	1.8^{B}	2.0^{B}	2.0^{B}	0.2	0.988
2	1.0^{C}	0.9^{C}	1.0^{B}	0.8^{C}	1.0^{C}	0.9^{C}	0.2	0.952
3	0.8^{C}	0.8^{C}	0.9^{B}	0.7^{C}	1.0^{C}	0.8^{C}	0.2	0.794
SE[2]	0.2	0.2	0.4	0.1	0.2	0.2		
p value[2]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		
	Crude Fibre (CF), %							
Initial	29.0	29.2	30.3	29.5	30.0	30.2	0.6	0.531
1	30.7	30.0	30.0	30.4	29.8	29.9	0.7	0.942
2	29.9	29.4	29.3	29.9	30.7	30.2	0.5	0.385
3	30.7	30.5	30.0	30.1	29.9	29.7	0.4	0.492
SE[2]	0.9	0.6	0.4	0.5	0.5	0.4		
p value[2]	0.438	0.403	0.438	0.685	0.533	0.809		
	Ash, %							
Initial	11.6^{a}	10.0^{Bb}	9.9^{Bb}	10.1^{Bb}	11.2^{a}	10.1^{Bb}	0.2	<0.001
1	11.6^{a}	10.9^{Aab}	10.1^{Bb}	10.8^{ABab}	11.7^{a}	10.2^{Bb}	0.4	0.039
2	11.9^{ab}	10.8^{ABbc}	10.6^{ABc}	11.1^{ABbc}	12.2^{a}	11.2^{Aabc}	0.3	0.002
3	12.1^{ab}	11.1^{Ac}	11.0^{Ac}	11.4^{Abc}	12.4^{a}	11.9^{Aabc}	0.2	0.001
SE[2]	0.4	0.2	0.2	0.3	0.4	0.2		
p value[2]	0.656	0.014	0.011	0.049	0.137	<0.001		

a, b, c mean values were significantly ($p < 0{\cdot}05$) different within the row across treatments
A, B, C mean values were significantly ($p < 0{\cdot}05$) different within the column across the storage period
[1]Standard error (SE) and P-value across the treatments
[2]SE and P-value across the storage periods

Gadeken and Casper (2017) reported 3.4% and 3.0%, respectively EE in high and medium forage TMR fresh rations.

Kulathunga et al. (2015) reported 0.3 – 1.5% EE in the feed blocks prepared with industrial by-products whereas

Santhiralingam and Sinniah (2018) reported 3.58 – 5.47% of EE in the complete feed blocks. These variations may be due to the various ingredients used in the preparation of feed blocks. However, the reported values in this study matched with the NRC (2001) recommendations as there is a limitation in incorporating dietary fat to dairy cow rations where a maximum level of 6 to 7% fat on DM basis is the recommended level (Humer et al. 2018).

Ash content

Ash content in T2, T3, T4 and T6 significantly increased across periods compared to the initial ash content (Table 3).

The total mineral content of the feed mixture is comprised of plant-derived minerals such as Ca, P, K , Mg and, minerals associated with soils such as silica and supplemental minerals such as premixes, salt, and buffers (Hoffman 2005). Accordingly, Hoffman (2005) observed an ash content of 9-17% in 1000 different TMR. The ash contents of the present study are within the above range. During the ashing processes, organic matter and minerals in organic combination are oxidized to inorganic form, hence the measurement of ash content does not provide any information regarding any specific elements present in the given biological sample (Fuller 2004). Therefore, the differences reported in treatments may be attributed to the different compositions of ingredients and level of contamination.

Crude Fibre (CF), Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF) contents

The CF, NDF and ADF contents were not significantly differed among the treatments within and across periods (Table 3 and 4), except for NDF content in T2.

Gadeken and Casper (2017) reported 33.6% & 30.5% NDF and 24.2% & 22.0% ADF in medium and high forage TMR fresh rations, respectively. Cows need more than 50% of the fibre in the diet to maintain chewing activity, ensure proper rumen health and to maintain the fat content in milk (Zebeli et al. 2012). The NRC (2001) recommends a minimum of 19% ADF and 30% NDF in a ration on DM basis, respectively out of which a minimum of 21% should be available from forage sources for milking cows. Accordingly, all the TMR rations formulated in this study satisfy the NRC (2001) requirements.

Table 4. Other nutrient composition of total mixed ration briquettes in three months storage

Month	Treatments						SE[1]	*p* value[1]
	1	2	3	4	5	6		
	Nutrient Detergent Fibre (NDF), %							
Initial	34.1	35.5^{A}	35.0	35.1	35.1	35.4	0.4	0.249
1	34.4	34.7^{AB}	35.0	34.8	35.2	34.9	0.2	0.147
2	34.5	34.8^{AB}	34.9	34.2	34.8	35.0	0.2	0.069
3	34.1	34.4^{B}	34.7	34.5	34.6	34.7	0.2	0.345
SE[2]	0.1	0.2	0.4	0.3	0.2	0.1		
p value[2]	0.156	0.039	0.962	0.283	0.350	0.072		
	Acid Detergent Fibre (ADF), %							
Initial	23.3	24.8	25.1	23.8	26.3	24.7	0.8	0.274
1	23.2	24.1	25.6	26.6	26.0	26.2	1.0	0.277
2	23.3	23.3	24.7	25.7	26.7	25.5	0.7	0.076
3	23.6	24.1	25.3	25.4	26.2	25.5	0.8	0.333
SE[2]	0.7	0.7	1.4	0.6	0.6	0.7		
p value[2]	0.979	0.568	0.963	0.128	0.895	0.595		
	Nitrogen Free Extract (NFE), %							
Initial	45.9	47.4	45.6	47.2	44.6	45.8	0.7	0.082
1	45.3	45.6	46.1	46.0	45.0	46.8	0.9	0.769
2	45.8^{ab}	47.0^{a}	47.2^{a}	47.2^{a}	44.0^{b}	46.4^{ab}	0.6	0.008
3	45.8	46.9	46.6	46.6	46.0	46.8	0.5	0.601
SE[2]	1.0	0.7	0.5	0.7	0.7	0.6		
p value[2]	0.970	0.248	0.222	0.565	0.286	0.631		
	Non-Fibre Carbohydrate (NFC), %							
Initial	40.8	41.1	40.8	40.8^{A}	39.1	40.4	0.5	0.169
1	42.3	40.3	40.8	41.7^{AB}	38.7	41.9	0.8	0.120
2	40.8^{ab}	41.2^{ab}	42.0^{ab}	42.5^{Ba}	39.8^{b}	41.0^{ab}	0.4	0.039
3	41.7	42.3	42.0	43.0^{B}	41.0	42.2	0.4	0.130
SE[2]	0.6	0.6	0.5	0.3	0.7	0.7		
p value[2]	0.099	0.226	0.244	0.017	0.253	0.386		
	Calcium (Ca), %							
Initial	0.56^{bc}	0.35^{d}	0.63^{ab}	0.37^{cd}	0.77^{a}	0.40^{ABcd}	0.03	0.001
1	0.59^{ab}	0.33^{b}	0.61^{ab}	0.41^{b}	0.75^{a}	0.40^{ABb}	0.06	0.014
2	0.66^{a}	0.40^{b}	0.61^{ab}	0.43^{ab}	0.62^{ab}	0.38^{Bb}	0.04	0.011
3	0.70^{ab}	0.44^{ab}	0.72 ^{a}	0.40^{b}	0.48^{ab}	0.63^{Aab}	0.06	0.021
SE[2]	0.07	0.03	0.05	0.02	0.06	0.04		
p value[2]	0.538	0.124	0.465	0.339	0.076	0.042		
	Phosphorous (P), %							
Initial	0.42^{b}	0.34^{b}	0.51^{ab}	0.35^{Ab}	0.79^{Aa}	0.34^{b}	0.06	0.011
1	0.58^{ab}	0.35^{b}	0.54^{ab}	0.34^{Ab}	0.66^{ACa}	0.35^{b}	0.06	0.031
2	0.44^{a}	0.30^{bc}	0.36^{abc}	0.26^{Bc}	0.41^{BCab}	0.26^{c}	0.03	0.008
3	0.53^{a}	0.28^{b}	0.44^{ab}	0.26^{Bb}	0.32^{Bab}	0.45^{ab}	0.04	0.017
SE[2]	0.08	0.03	0.04	0.01	0.06	0.04		
p value[2]	0.551	0.370	0.072	0.010	0.011	0.154		
	Magnesium (Mg), %							
Initial	0.22^{ab}	0.22^{ab}	0.23^{b}	0.19^{b}	0.25^{Aa}	0.21^{b}	0.01	0.009
1	0.23^{ab}	0.21^{ab}	0.20^{b}	0.20^{b}	0.26^{Aa}	0.20^{b}	0.01	0.016
2	0.25^{a}	0.22^{ab}	0.21^{ab}	0.20^{b}	0.23^{Aab}	0.21^{b}	0.01	0.020
3	0.25^{a}	0.22^{ab}	0.21^{bc}	0.19^{bc}	0.17^{Bc}	0.20^{bc}	0.01	0.002
SE[2]	0.01	0.01	0.01	0.00	0.01	0.01		
p value[2]	0.158	0.898	0.513	0.410	0.012	0.381		

a, b, c mean values were significantly ($p < 0{\cdot}05$) different within the row across treatments

A, B, C mean values were significantly ($p < 0{\cdot}05$) different within the column across the storage period

[1]Standard error (SE) and P-value across the treatments

[2]SE and P-value across the storage periods

Table 5. Energy composition of total mixed ration briquettes in three months storage

Month	Treatments						SE[1]	*p* value[1]
	1	2	3	4	5	6		
	Total Digestible Nutrient (TDN), %							
Initial	63.3^{A}	64.7^{A}	65.1^{A}	64.0^{A}	63.9^{A}	64.1^{A}	0.6	0.427
1	60.1^{B}	61.4^{B}	62.3^{AB}	61.0^{B}	60.9^{B}	62.1^{A}	0.7	0.271
2	59.1^{Bab}	60.2^{BCa}	60.7^{Ba}	59.5^{BCab}	58.4^{Cb}	59.4^{Bab}	0.4	0.008
3	57.9^{Bb}	59.2^{Cab}	59.8^{Ba}	58.9^{Cab}	58.4^{Cab}	58.8^{Bab}	0.4	0.050
SE[2]	0.6	0.3	0.7	0.4	0.4	0.5		
p value[2]	0.001	<0.001	0.001	<0.001	<0.001	<0.001		
	Metabolizable Energy (ME), kcal/kg							
Initial	2374^{A}	2437^{A}	2455^{A}	2408^{A}	2405^{A}	2413^{A}	27.4	0.419
1	2235^{B}	2291^{B}	2330^{AB}	2274^{B}	2271^{B}	2324^{A}	30.0	0.270
2	2188^{Bab}	2239^{BCa}	2261^{Ba}	2207^{BCab}	2157^{Cb}	2197^{Bab}	16.7	0.006
3	2136^{Bb}	2192^{Cab}	2221^{Ba}	2181^{Cab}	2157^{Cab}	2174^{Bab}	18.1	0.049
SE[2]	28.5	14.9	32.4	20.0	19.9	22.8		
p value[2]	0.001	<0.001	0.001	<0.0001	<0.001	<0.001		
	Gross Energy (GE), kcal/kg							
Initial	3733^{Ab}	3749^{b}	3804^{Aab}	3733^{Ab}	3830^{Aa}	3761^{Aab}	16.5	0.002
1	3728^{A}	3745	3771^{AB}	3726^{A}	3767^{B}	3728^{A}	10.9	0.051
2	3610^{Bb}	3703^{a}	3712^{Ba}	3659^{Bab}	3723^{Ba}	3710^{Aa}	19.0	0.004
3	3609^{B}	3693	3629^{C}	3641^{B}	3665^{C}	3644^{B}	22.3	0.183
SE[2]	18.7	19.0	18.4	22.6	11.7	13.7		
p value[2]	<0.001	0.134	<0.001	0.029	<0.001	0.001		

a, b, c mean values were significantly ($p < 0{\cdot}05$) different within the row across treatments
A, B, C mean values were significantly ($p < 0{\cdot}05$) different within the column across the storage period
[1]Standard error (SE) and P-value across the treatments
[2]SE and *p*-value across the storage periods

Calcium (Ca), Phosphorous (P) and Magnesium (Mg) contents

As indicated in Table 4, by three months of storage, the significantly highest Ca content was observed in T3 compared to T4, and the highest P and Mg contents were observed in T1 compared to T2 and T5, respectively.

The sufficient dietary requirement of Ca prevents osteoporosis and milk fever while sufficient dietary P allowance enhances the growth rate of the animals (Freer et al. 2007). Ibrahim (1988), indicates that the dietary Ca% and P% requirements for dairy cows are 0.45% and 0.40%, respectively under local conditions. Accordingly, TMR recipe briquettes in the present study have the required dietary Ca and P levels. Magnesium is also an important mineral that has to be provided with the diets of ruminants, because it acts as a cofactor for many enzymes. Accordingly, Ibrahim (1988) indicates that the dietary Mg requirement for dairy cows ranged between 0.14% to 0.35% and the Mg levels in the present TMR recipe briquettes are in the recommended range.

Total Digestible Nutrients (TDN), Metabolizable Energy (ME) and Gross Energy (GE)

Total digestible nutrients and ME in six TMR recipes were not significantly different within the first month (Table 5). Gross energy content in all treatments did not significantly change at 3 months of storage. Total digestible energy and ME contents in all the treatments reduced ($p < 0.05$) across periods compared to the initial TDN and ME contents but within the acceptable range.

Shelf Life of the TMR Briquettes

Yeast and Mould Counts (Y&MC)

There was no difference ($p > 0{\cdot}05$) in yeast count across periods except T5 in which yeast count has been reduced (Table 6).

Mould counts were not different ($p > 0{\cdot}05$) within the period across treatments except for period 3 (Table 6). There was no difference ($p > 0{\cdot}05$) in mould count across periods in T1. However, other treatments had significantly higher mould counts across periods compared to the initial mould count. Similar results have been reported in a previous study conducted on a TMR prepared with local feed ingredients in Mozambique (Du et al. 2020).

In the present study, T4 resulted the highest Y&MC while T1 reported the lowest values after 3 months of storage. Treatment 4 contained molasses and it may have contributed to higher Y&MC as the molasses can be contaminated with moisture. Previous research has shown moisture content created ideal conditions for the rapid growth of moulds and mycotoxin production (Van der Heijden and de Haan 2010; Santhiralingam and Sinniah 2018). Generally, the upper limit for yeast and mould enumeration counts of a TMR ration is 13.6×10^6 $CFUg^{-1}$ and 4×10^5 $CFUg^{-1}$, respectively (Goeser 2016). Therefore, the yeast and mould values obtained in the current study were within these standards in all six treatments during the entire storage period.

Total Plate Counts (TPC)

Total plate counts were significantly higher in T4, T5 and T6 compared to other treatments at the initial period (Table 6). Total plate counts were not different ($p > 0{\cdot}05$) within T2 and T3 across periods. However, in other treatments, TPC were significantly increased during the period, but within the acceptable range.

Free Fatty Acids (FFA)

Free fatty acids content was the highest in T2 while the lowest was observed in T6 in the first month of storage (Table 6). At the end of three months of storage, T5 had the least amount of FFA content (0.19%) compared to the other treatments and it was significantly different compared to T4 and T6. Free fatty acid contents were significantly lower across periods within treatments.

Free fatty acids are the freely available fatty acids which are not bound to any lipids/fats. Measurement of FFA content is an important indication of the shelf life of the feed since the shelf life of a feed get reduced when the FFA percentage is high. It is a measurement of rancidity and it will be affected by light intensity, heat and moisture content and the presence of traces of metals such as copper and iron (Pearson 1973). Free fatty acid calculations vary depending on the type of oil and Pearson (1973) has taken 1% FFA level as a guide for human consumption. Somasiri et al. (2010) has considered 5% FFA level as the critical level to select the best leguminous leaf meal block recipe prepared for cattle feeding. In the present study, FFA content was calculated based on Linoleic acid and it was less than 1.0% in all the TMR recipe briquettes. Thus, according to FFA content, the TMR recipe briquettes in the present study are at the acceptance level (Pearson 1973). In contrast, Santhiralingam and Sinniah (2018) observed a higher FFA level (4.1 - 11.7%) from feed blocks made from different combination of local fodder grasses and agricultural waste. By the end of three months, FFA levels were further reduced compared to its initial levels. Thus, by proper packaging and maintaining proper storage temperatures, fat decomposition could be further reduced (Somasiri et al. 2010).

Presence of Potentially Toxic Metals in TMR Briquettes

The proportion of the potentially toxic metals in all the TMR recipe briquettes were as follows; Cd (0.07±0.06 - 0.83±0.13 ppb), Pb (40.94±5.28 – 79.56±13.30 ppb), As (0.29±0.05 – 1.35±0.11 ppb) and Hg (not detectable) within and across periods. According to Suttle (2010), the maximum permitted level and maximum tolerable level for Cd, Pb, As and Hg in complete ruminant feeds are; 1 and 10 Cd, 5 and 100 Pb, 2 and 30 As and 0.1 and 2 Hg mgkg^{-1} DM basis, respectively. Hence, the values observed in the present study were within the acceptable levels minimizing the risk associated with investigated toxic metals.

Table 6. Microbial count and free fatty acid content in total mixed ration briquettes in three months storage

Month	Treatments 1	2	3	4	5	6	SE[1]	*p* value[1]
	Yeast, log_{10} cfu/g							
Initial	4.29^{b}	4.37^{b}	4.52^{ab}	4.79^{a}	4.40^{Bb}	4.51^{ab}	0.06	0.009
1	4.37^{b}	4.46^{b}	4.62^{ab}	4.89^{a}	4.60^{ABab}	4.57^{ab}	0.08	0.029
2	4.39^{b}	4.53^{ab}	4.72^{ab}	4.87^{a}	4.67^{Aab}	4.61^{ab}	0.07	0.025
3	4.47^{b}	4.55^{ab}	4.77^{ab}	4.96^{a}	4.71^{Aab}	4.65^{ab}	0.08	0.038
SE[2]	0.09	0.09	0.04	0.06	0.04	0.08		
p value[2]	0.624	0.533	0.055	0.322	0.015	0.712		
	Moulds, log_{10} cfu/g							
Initial	3.74	3.81^{B}	3.74^{B}	3.87^{C}	3.81^{B}	3.87^{B}	0.03	0.099
1	4.00	4.04^{AB}	4.02^{A}	4.16^{B}	4.06^{A}	4.15^{A}	0.04	0.145
2	4.05	4.15^{A}	4.06^{A}	4.22^{AB}	4.13^{A}	4.20^{A}	0.05	0.202
3	4.09^{b}	4.24^{Aab}	4.11^{Ab}	4.37^{Aa}	4.20^{Aab}	4.31^{Aab}	0.04	0.018
SE[2]	0.06	0.04	0.04	0.03	0.03	0.04		
p value[2]	0.053	0.009	0.011	0.002	0.001	0.005		
	Total Plate Count (TPC), log_{10} cfu/g							
Initial	4.70^{Cbc}	4.53^{d}	4.58^{dc}	4.76^{Bab}	4.84^{Ca}	4.84^{Ba}	0.03	<0.001
1	4.79^{BCa}	4.57^{b}	4.64^{b}	4.88^{ABa}	4.91^{BCa}	4.92^{ABa}	0.03	<0.001
2	4.85^{Bb}	4.59^{c}	4.70^{c}	4.94^{Aab}	4.96^{Aba}	4.97^{Aa}	0.03	<0.001
3	4.96^{Aa}	4.67^{b}	4.74^{b}	4.98^{Aa}	5.03^{Aa}	5.02^{Aa}	0.05	<0.001
SE[2]	0.03	0.04	0.05	0.03	0.03	0.03		
p value[2]	<0.001	0.086	0.178	0.001	0.002	0.004		
	Free Fatty Acids (FFA), %							
Initial	0.80^{Aabc}	0.69^{Ac}	0.82^{Aab}	0.71^{Abc}	0.74^{Aabc}	0.84^{Aa}	0.02	0.013
1	0.39^{Bab}	0.52^{Ba}	0.25^{Bbc}	0.28^{Bbc}	0.33^{Bbc}	0.22^{Bc}	0.03	0.002
2	0.27^{Bab}	0.22^{Cb}	0.22^{Bb}	0.30^{Bab}	0.35^{Ba}	0.21^{Bb}	0.02	0.008
3	0.27^{Babc}	0.20^{Cc}	0.24^{Bbc}	0.36^{Ba}	0.19^{Cc}	0.34^{Bab}	0.02	0.003
SE[2]	0.02	0.02	0.03	0.02	0.02	0.02		
p value[2]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		

a, b, c mean values were significantly ($p < 0{\cdot}05$) different within the row across treatments
A, B, C mean values were significantly ($p < 0{\cdot}05$) different within the column across the storage period
[1]Standard error (SE) and *p*-value across the treatments
[2]SE and *p*-value across the storage periods

Cost Analysis of Different TMR Briquettes

Animal feed costs are shown to be the largest single item of cost in livestock production. The proposed option of producing TMR recipes using locally available ingredients seemed at easing the impacts of higher feed costs while providing nutritionally balanced feed requirements for lactating cows. The cost analysis was performed to find out the most cost effective TMR recipe among the six different TMR recipes. The results revealed that the cost of production of 1 kg of TMR briquette ranges from Rs 28.40 to Rs 33.15, having the least cost of production for T5 (Rs. 28.40) and the highest for T3 (Rs. 33.15). The cost of production of T6 (Rs. 29.01) was lower than the cost of production of T1 (Rs. 32.48), T2 (Rs. 30.24), T3 and T4 (Rs. 29.78), but higher than T5. Higher amounts of maize meal and SBM in T3 seem to incur the most incremental cost compared to T5 and T6. The mineral mixture was the largest cost contributor in both of these recipes that accounts for about 19% of the cost-share. Coconut poonac was the second largest cost contributor accounting for a 16% share having higher amounts of this ingredient in T5 and T6 compared to T3. Coconut poonac is highly available locally for the use of animal feed industry compared to maize meal and SBM. Hence, the findings reconfirm the feasibility of using T5 and T6 recipes as the most cost-effective option for making TMR briquettes.

4. Conclusion

The total mixed ration recipe briquettes prepared in the present study is successful with respect to the measurements of physical characters, nutritional composition, shelf life and cost of production. All the TMR recipe briquettes could be stored for up to three months without any deterioration of nutritional composition, adverse changes in physical characters and significant microbial growth. Hence, depending on the cost of production T5 and T6 TMR briquettes were selected as the best two potential recipes for further studies. It is expected to undertake a feeding trial as the next step to decide on the best TMR recipe briquette among the selected two recipes.

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