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Effects of feeding ground versus pelleted total mixed ration on digestion, rumen function and milk production performance of dairy cows

Running head: Ground versus pelleted total mixed ration in dairy cows

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32 **ABSTRACT**

33 *This study evaluated the effects of feeding ground (G) versus pelleted (P) total mixed ration*
34 *(PTMR) on digestibility and milk production performance of mid-lactation dairy cows over a 42-d*
35 *period. Results showed that, compared to GTMR, feeding PTMR increased dry matter intake and*
36 *digestibility, ruminal propionate proportions and milk protein percentage, but decreased acetate*
37 *to propionate ratio, milk fat percentage and aldehydes, acids, alcohols, and aromatic*
38 *hydrocarbons proportions in milk. The PTMR did not improve milk yield but accelerated live*
39 *weight recovery of cows and led to acidosis risk in lactating cows due to negative effects on rumen*
40 *fermentation.*

41 **INTRODUCTION**

42 Dairy cattle play an important role in global food security; world milk production reached 811
43 million tonnes in 2017, 1.4% higher than in 2016 (FAO 2018). However, due to higher population
44 growth rates and changes in land use, available land for forage production is declining annually.
45 Therefore, extensive use of feed resources and advanced feed technologies are needed to maximize
46 effective utilization rates of available feed resources to improve profitability and sustainability.

47 Before 1960s, dairy herds worldwide were mostly housed in stanchion or tie-stall barns and
48 fed concentrate and forages separately. When individual feed components were fed separately,
49 cows often sort their feed and can adjust their intake of various feed component. They often sort
50 for short particles and against long particles when fed a low-forage diet, which leads to a lower
51 amount of neutral detergent fiber (NDF) consumed compared to that when fed a high-forage diet,
52 which sometimes led to digestive upsets (Eastridge 2006). Given greater demands for milk and
53 increasing milk production from individual cows, diets with balanced nutrients and economic
54 feeding are increasingly important.

55 Total mixed rations (TMR) were introduced in the last half-century. One of the earliest
56 studies (McCoy *et al.* 1966) indicated that feeding TMR as opposed to feeding forages and
57 concentrates separately provided an opportunity to make every mouthful of feed essentially a
58 complete, nutritionally balanced diet for animals. Comprehensive advantages and disadvantages of
59 TMR were thoroughly reviewed by Schingoethe (2017). Tiny particles in the ground TMR
60 (GTMR) are easily inhaled into the nostrils of cows which can lead to pneumonia.

61 Pelleted total mixed rations (PTMR) have been used in intensive feeding systems for sheep
62 (Zhong *et al.* 2018), goats (Chen *et al.* 2018) and heifers (Bonfante *et al.* 2016). The PTMR are
63 generally comprised of all roughages (forages, crop residues and by-products), concentrates,

minerals, and vitamins, which are formulated to meet total nutrient requirements for animals in a pelleted form. Comparing with GTMR, the use of PTMR not only offers many practical advantages, including full use of local feed resources (including agro-industrial byproducts, crop residues, and unconventional non-palatable feed resources), it saves storage spaces and transportation costs and minimizes labor, but also exerts both positive physiological benefits and negative effects, including increasing growth performance and dressing percentage of ruminants but reducing chewing time (Islam *et al.* 2017). However, the major drawback of PTMR is higher processing cost for hard and long-fiber forages; another drawback is lower rumen pH due to lack of physically effective NDF and high percentage of concentrates (Zhong *et al.* 2018). To reduce cost of processing forages, partial long fiber forages were substituted by high-fiber crop residues and by-products, which were always used as fiber-providing ingredients in PTMR. Until now, there are limited data regarding whether feeding PTMR, containing low forage with short or fine particle fibers, is feasible in dairy cows.

Therefore this study aimed to determine the effects of feeding GTMR versus PTMR on bodyweight change, nutrient digestibility, rumen function, blood metabolites, and milk production/components in mid-lactation cows. We hypothesized that feeding PTMR might mainly affect cows' milk yield and components and would impair rumen fermentation.

MATERIALS AND METHODS

Animals and experiment design

The work was approved by Animal Care Committee of the Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun, Jilin, China.

Twelve multiparous (2nd parity) Holstein cows at 100 ± 7.0 (mean $\pm$ SD) days of lactating with average live weight of 765 ± 22.4 (mean $\pm$ SD) kg were randomly assigned using Microsoft Excel 2010 to two dietary treatments (6 cows per treatment) using a randomized design based on days of lactating and live weight. Dietary treatments were GTMR and PTMR. All diets were made according to NRC (2001) requirements for mid-lactation cows (Table 1) using BRILL (1.3008, USA) nutrition software. The study consisted of a 14-d feed adaptation period (all cows fed high forage TMR) and a 42-d measurement period. During feeding, all diets were supplied individually twice daily at 0800 and 2000 h, with feed constantly available andorts less than 5 % of the supplied feed. During the study, all cows were held in a free stall barn individually. Each cow was housed in individual pens (3.0 m $\times$ 5.0 m) to ensure cows were fed individually. All cows had free access to water and were milked three times daily at 0700, 1600, and 2400 h using an automatic milk machine (model HP101 milking machine, Alfa Laval Agricultural Corp., Guangzhou, China),

equipped with a milking recorder cup.

Preparing total mixed rations

Maize stalks were harvested at full ripe period and the silage was produced 3 months prior to the start of the study using fresh harvested maize stalks without cobs that was, chopped, and shredded (3-4 cm length). Alfalfa hay was similarly chopped and shredded (3-4 cm length). Alfalfa hay for GTMR and PTMR was ground to pass through a 0.5-cm screen. Some of the ground alfalfa hay was mixed with concentrate ingredients using a mixer machine to prepare GTMR (DSH, Zesen Machinery, Shandong, China) before feeding. Sufficient GTMR for one week study was made weekly in one batch. Some of the ground hay was mixed with concentrate ingredients and then pelleted into PTMR (GSL, Changzhou Ou Drying Equipment Co., Ltd., China) with steam added at a 75 °C exit temperature. Sufficient PTMR for the entire study was made in a single batch. Yield of PTMR averaged 2-4 tons per hour (diameter = 4.2 mm). All concentrate ingredients for TMR were ground through a 5.0 mm (for corn) or a 4.0 mm (for all other ingredients) screen and well mixed. The chemical composition of feedstuffs, including dry matter (DM), crude protein (CP), NDF, acid detergent fiber (ADF), ether extract, starch, ash, Ca, and total P content, were determined in lab of Institute of Geography and Agroecology according to methods of (AOAC 1990) and (Van Soest *et al.* 1991). The NE_L value was calculated using NRC (2001).

Sampling and analysis

Every time feed was supplied for each cow it was recorded and the orts of each cow were manually weighed before providing feed for next time. The PTMR samples (500 g) were collected on the first day of the feeding period. The GTMR samples (500 g) were collected before feeding on days, 0, 14, 28, 35, and 42 of the measurement period by Quadruple Method (Yang 1993). The feed DM was determined by drying samples at 65 °C to a constant weight. Prior to the morning feeding on measurement days 0, 14, 28, and 42, all cows were weighed by an automated walkover cattle weighing scale (SCS model, Tianjin Hongzhong Electronic Weighing Apparatus Co., LTD, Tianjin, China) and average daily gain (ADG) was calculated as the slope of the linear regression of BW throughout time. Feed conversion ratio (FCR) was calculated by taking average DMI of each cow divided by ADG.

Milk production from individual cow was quantified daily and a 100 ml sub sample from per cow was collected at each daily milking on days 0, 7, 14, 28, 35, and 42 of the measurement period. When sampling, twice a day milk samples for each cow were stored at 4 °C before compositing equally with evening samples. Compositions of sub-composited samples were analyzed immediately with an automatic milk analyzer with mid-infrared wave-band procedures (MILKYWAY-2, Zhejinag University, Jiangsu, China). The fat-corrected milk (FCM) was

calculated as follows: FCM = milk yield $\times$ (0.4 + 0.15 fat content). Remaining sub-composited samples were stored at -80 °C for analysis of volatile compounds using headspace solid-phase microextraction-gas chromatography-mass spectrometry (Aunsbjerg *et al.* 2015).

On measurement days 0, 14, 28, and 42, blood samples (5 ml) were harvested from coccygeal vessels per cow before morning milking into Lith-heparin tubes and then centrifuged immediately at $2500 \times g$ for 15 minutes at 4 °C. Plasma was harvested and stored at -20 °C until analysis. Plasma biochemical parameters were analysed using a blood analyzer (Hitachi, 7020, Beijing, China) and commercial kits (Jiancheng Biology Co., Nanjing, China).

Ruminal samples were harvested per cow via oral lavage (Lodge-Ivey *et al.* 2009) at 2 hours after the morning feeding on the last day of the measurement period. Ruminal pH was measured using a pH meter (Testo 205, Testo AG, Lenzkirch, Germany). Thereafter, ruminal fluid was strained through 4 layers of cheesecloth and strained samples were stored at -20 °C before the measurements of ammonia nitrogen (NH₃-N) (using phenol-hypochlorite; Broderick and Kang 1980) and volatile fatty acid (VFA) (using gas chromatography; Isac *et al.* 1994).

Feed nutrient digestibility was calculated per description of Schäfers *et al.* (2018), chromium oxide (Cr₂O₃) was fed to cows as an external marker. The digestibility trial was conducted from measurement days 36 to 42. Briefly, Cr₂O₃ was mixed with GTMR and PTMR at a ratio of 0.5% of the feed (DM basis). Sufficient PTMR with Cr₂O₃ for entire experiment was produced prior to the study. Approximately 100 g fresh feces per cow were collected for 7 consecutive days at 2, 4, 6, 8, and 12 hrs post-feeding. All daily feces from each cow were pooled and stored at -20 °C. Sampled feeds and feces of each cow over 7 days were thawed, subsampled, dried at 65 °C for 48 h to determined DM, ground through a 1-mm screen, and stored at 4 °C for chemical analysis. Total content of ether extract from diets and feces were determined by ether extraction. The N content of diets and feces were measured by the method of AOAC (1990). The content of NDF and ADF were determined according to method as described by Van Soest *et al.* (1991). Starch content was analysed per procedure as described by Poore *et al.* (1991). Chromium content in feces and feed was analyzed as described by Schäfers *et al.* (2018).

Statistical analyses

The DM intake, apparent digestibility of feed DM and nutritive value, and rumen fermentation of cows were analyzed by a General Linear Model (SAS 2002). Data for blood, milk production, and milk composition were analyzed by a MIXED model procedure of SAS (SAS Institute Inc., Cary, NC; Littell *et al.* 1996). The model consisted of diet treatment, feeding day, treatment $\times$ feeding day interactions as fixed effects and cow as the random effect. Measurements obtained per cow at various sampling times were treated as repeated measures. Means were separated using the least

squares mean and the differences were declared when $P \leq 0.05$.

RESULTS

Feed intake, nutrient digestion and rumen fermentation

The DMI was increased ($P < 0.001$) 2.59 %, the ADG was increased ($P < 0.001$) 52.08 %, and the FCR was decreased ($P < 0.001$) 33.76 % by PTMR, compared to GTMR cows (Table 2). Feeding different physical forms of TMR resulted in many differences in nutrient digestion. Compared to GTMR, apparent digestibility of DM ($P = 0.013$), CP ($P = 0.004$), ether extract ($P = 0.008$), and starch ($P = 0.003$) were increased by feeding PTMR. For the rumen parameters, despite no changes in total VFA concentration between two treatments ($P = 0.857$), compared to GTMR, PTMR decreased $\text{NH}_3\text{-N}$ concentration by 17.2% ($P = 0.016$), ruminal pH ($P < 0.001$), and acetate molar proportion by 8.8 % ($P < 0.001$), but it increased molar proportions of propionate by 20.3 % ($P < 0.001$) and reduced the ratio of acetate to propionate ($P < 0.001$). However, no clinical signs of acidosis have been observed for all cows fed with PTMR.

Blood metabolites

No significant interaction between treatment and feeding time was found from metabolites in plasma (Table 3). Dietary treatments did not affect all blood metabolites of cows. The plasma urea nitrogen (PUN) and glucose concentrations were increased with the extension of the feeding days ($P = 0.010$). Plasma glucose concentrations changed with the extension of the feeding days ($P = 0.032$).

Milk production and compositions

Compared to GTMR, milk yield for PTMR cows decreased ($P = 0.021$) by 3.6 % (Table 4). Moreover, milk yield for PTMR cows decreased ($P < 0.001$) from 38.47 kg/d to 34.49 kg/d over feeding time. An interactive effect ($P = 0.039$) was found between dietary treatment and feeding day on milk fat yield ($P = 0.012$) and protein percentage ($P = 0.002$). As to effect of feeding days on milk, yields of milk SNF ($P = 0.001$), lactose ($P = 0.002$) and ash ($P = 0.006$) were decreased from 3.24 kg/d to 3.01 kg/d, 1.83 kg/d to 1.65 kg/d, and 0.26 kg/d to 0.24 kg/d, respectively, over feeding time.

Volatile flavor compounds in milk

A total of 28 volatile flavor compounds were quantified in both treatments (Table 5). The GTMR milk contained 23 volatile organic compounds, with PTMR milk having only 18. In respect to composition of total volatile compounds, proportions of aldehydes and acids for PTMR milk were lower than that of for GTMR ($P < 0.05$), whereas proportion of aromatic hydrocarbons for PTMR

milk was numerically (non-significant) higher compared with that for GTMR milk (Figure 1).

DISCUSSION

Mid-lactation occurs between 100 to 200 days after calving, the main production purposes of this stage are to maintain peak milk production as long as possible and reduce weight loss to promote rapid postpartum estrus. The key strategy for increasing milk production is to maximize DMI. Sorting behavior is an important factor influencing DMI of dairy cows. Feed sorting by cows is contributed by forage inclusion, particle size, and DM%. In the present study, increased DMI for PTMR cows might be related to the reduced dietary fiber particle size in PTMR, as pelleted form of diet takes up lower rumen volume than GTMR (Yang and Beauchemin 2007). Accordingly, increased DMI and DM digestibility for PTMR cows resulted in a higher ADG and a lower FCR, which was consistent with findings when a PTMR was fed to meat sheep (Islam *et al.* 2017). The cost associated with pelleting TMR was 20 US dollar per ton feed, including manual work, water, electricity, and machinery depreciation, but more costs associated with transportation, storage, and labor were accordingly saved by using PTMR in this study. Cows fed with GTMR did not get pneumonia. Cows also had no clinical signs of acidosis when fed with PTMR. These results indicated that PTMR may likely be well accepted by dairy cow industry.

Physical forms of a diet affect its feeding value. Generally, feeding TMR increases dietary NDF digestibility compared to feeding forage and concentrate separately (Hundal *et al.* 2004). Feeding PTMR would further increase dietary NDF digestibility compared to that of non-pelleted TMR, due to shorter fiber particle size which influences chewing time, saliva secretion, and retention time without causing an adverse effect on rumen fermentation for at least a short period (Bonfante *et al.* 2016). Pelleting affects rumen degradation of starch, protein, and fiber as previously showed by Castillo *et al.* (2006). Feeding PTMR increased digestibility of ether extract, NDF, and ADF in goats (Ananth and James 2013). We previously reported that feeding PTMR increased CP, ADF, ether extract, and starch digestibility in fattening lambs when compared to GTMR (Zhong *et al.* 2018). Potential reasons for increased DM, CP, and ether extract digestibility by PTMR in the present study may have been due to pelleting that not only resulted in a more gradual release of dietary fat in the rumen, but also promoted dietary CP to escape from rumen, which may increase bioavailability of dietary protein in ruminants. However, feeding PTMR decreased NDF and ADF digestibility, although not significantly; therefore, if fiber was finely ground, rumination would be depressed and fiber retention time in the gastrointestinal tract shortened, which may be detrimental to digestion. This inference was supported by the conclusion of Shaver *et al.* (1986), in which they demonstrated that nutrient digestibility in dairy cows would

be depressed at high intake due to shortened ruminal residence time.

Rumen function in cows is optimal when pH is within a range of 6.2 to 7.2. Although many metrics, such as VFA, milk production and components, and clinical signs have been used in combination to diagnose subacute ruminal acidosis (SARA), rumen pH < 5.8 is as a direct indicator of fibrolytic bacteria depression which may lead to lower fiber digestibility, and pH < 5.5 has been identified as one of the many indicators of SARA (Kleen *et al.* 2003). The decreased ruminal pH by PTMR may be closely related to higher concentrate in diets and reduced NH₃-N concentration, because high-concentrate diets decreased ruminal pH and increased propionate concentration and thus shifted rumen fermentation to propionate fermentation pattern in dairy goats (Desnoyes *et al.* 2008) and cows (Agle *et al.* 2010). The present study had similar effects of low forage based-TMR, irrespective of pelleting, on rumen fermentation of dairy cows. In the present study, ruminal pH values of cows in both treatments were > 6.0 and no clinical signs of SARA were observed in the current experiment. However, in this study, PTMR decreased rumen pH, acetate to propionate ratio, and NH₃-N concentration in rumen fluid, resulted in reduced milk yield and percentage ratio of milk fat to milk protein (1.05 vs 0.95 for GTMR vs PTMR). These factors all indicated a higher risk of acidosis in cows, but caution is needed as we only performed the analyzed based on one-time sample (42nd day). Therefore, more work is needed to evaluate long-term effects of feeding PTMR on lactating performance and health status of dairy cows.

Blood metabolics are routinely used to diagnose metabolic disorders in dairy cows (Gross *et al.* 2011). There is a lack of comprehensive conclusions from the limited data on changes in blood metabolic profile in ruminants fed PTMR. For example, blood urea nitrogen concentration decreased and total protein, albumin, as well as glucose concentrations increased by feeding rice straw based-PTMR in growing goats (Pi *et al.* 2005). Furthermore, although total protein, albumin, and globulin concentrations varied between arhar stalk and gram straw-based PTMR diets, blood metabolomics of goats were within the normal range (Rekhate *et al.* 2008). However, altering the form of feeds did not change blood metabolomics between GTMR-fed versus PTMR-fed sheep (Islam *et al.* 2017). In this study, dietary treatments did not cause significant effects of PTMR on blood metabolites, but PUN and glucose concentrations increased over feeding period. Similar to milk urea nitrogen (Kohn *et al.* 2002), PUN concentration may provide prediction of nitrogen excretion and nitrogen utilization of cattle (Kohn *et al.* 2005). Increased PUN concentration might be largely attributed to increased absorption of ruminal ammonia, which resulted in more ammonia being detoxified in the liver to form urea as lactation extends.

A meta-analysis based on 118 studies indicated that among some routinely measured parameters (DMI, BW, dietary forage proportion, and nutritive value of diets), DMI was the only

significant factor that affected milk component yield (Lee *et al.* 2014). Nagalakshmi and Reddy (2011) showed higher milk production and feed cost per kg FCM in buffaloes when they were fed PTMR (cotton stalks based), compared to non-pelleted diets. In this study, the PTMR-fed cows which had increased DMI compared to the GTMR-fed cows, and it resulted in a higher ADG, which was consistent with a report that feeding PTMR mainly affected carcass conformation and several physicochemical parameters, e.g. fatty acids profile, tenderness, and color of meat quality, of sheep (Ramírez-Retamal and Morales 2014).

Feeding various physical forms of TMR to dairy livestock can result in different milk composition. Milk fat and protein percentage are two of the most important indexes used by dairy companies and consumers to evaluate milk quality. Under non-pelleted TMR feeding conditions, there was higher SNF, protein, total solid and polyunsaturated fatty acid content in milk of buffaloes fed a TMR compared to when forage and concentrate were fed separately (Kumar *et al.* 2014). Feeding high-concentrate diets decreased milk fat percentage and increased milk protein percentage (Agle *et al.* 2010). Similar to these results, the present result of higher milk protein percentage by PTMR indicated benefits of feeding PTMR in dairy cows. However, Xie *et al.* (2017) reported that feeding high concentrate diets led to lower milk protein and fat percentage than feeding a low-concentrate diet. Under PTMR feeding conditions, Meel *et al.* (2016) reported that when compared to feeding non-pelleted TMR, feeding straw based-PTMR did not affect milk composition, although it increased DMI. The present increase in milk protein percentage was mostly due to there was an increase in propionate and reduction in ruminal NH₃-N concentration that enhanced milk protein synthesis (Hristov and Ropp 2003). In addition, pelleting may have caused a more gradual release of oil in the rumen, and a higher rumen escape of dietary CP, which can improve bioavailability of dietary CP and energy (Santos *et al.* 2011).

Flavor is contributed by a variety of volatile organic compounds (Lin *et al.* 2016). Both chemical structure and concentrations of volatile organic compounds are responsible for the smell. Volatile organic compounds such as hexanal, pentanal, and phenylacetaldehyde are associated with development of undesirable flavors and they have been proposed as potential markers of fresh milk quality (Karatapanis *et al.* 2006). Concentrations of hydrocarbons associate well with sensory flavor (Hall and Andersson 1985). Esters of short-chain fatty acids are responsible for fruity flavors that can be regarded as a defect by consumers (Liu *et al.* 2004). Diet can influence composition of volatile compounds in raw milk, either through rumen metabolism (Urbach 1990) or by transferring odour-active molecules (Buchin *et al.* 1999). However, there are limited data regarding milk flavor changes by PTMR. In the present study, PTMR significantly decreased aldehydes and acids contents, but numerically increased aromatic hydrocarbons and esters contents

of milk compared to that of the GTMR and no alcohols were detected in the PTMR milk. Therefore, all these effects indicated that feeding PTMR might be good for milk flavor improvement.

CONCLUSIONS

In the current study, although PTMR was well accepted by dairy cows in terms of their higher DMI and higher ADG, it could lead to a higher risk of acidosis in lactating cows due to negative effects on rumen fermentation over a 42-d period. Feeding PTMR decreased milk yield and milk fat percentage, but milk quality might be improved in terms of increased milk protein percentage and good flavor volatile compounds in milk. Further studies are needed to determine effectiveness of long-term feeding PTMR to dairy cows in various stages of lactation.

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CONFLICT OF INTEREST

The authors declared no conflicts of interest.

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1 **Table 1** Ingredient and chemical composition of adaptation and experimental diets.

Item ²	Adaptation diet	Experimental diets ¹	
	High forage TMR	GTMR	PTMR
Ingredient (% of DM)			
Maize silage	11.7	/	/
Steam-flaked corn	5.6	/	/
DDGS	7.6	/	/
Whole cottonseed	3.9	/	/
Molasses	3.1	/	/
Alfalfa hay	31.1	5.0	5.0
Ground corn	15.2	28.5	28.5
Soybean meal	11.9	14.3	14.3
Corn germ meal	3.9	21.8	21.8
Corn husk	/	14.3	14.3
Soybean hull	/	11.4	11.4
Ca-LCFA	1.6	0.7	0.7
Sodium bicarbonate	0.9	0.5	0.5
Minerals and vitamins salt ³	3.5	3.5	3.5
Chemical composition (% of DM)			
Dry matter (% of feed)	49.7	90.2	91.2
Crude protein	19.3	19.2	19.4
Neutral detergent fiber	34.1	35.8	36.0
Acid detergent fiber	26.4	28.7	29.7
Ether extract	8.6	7.1	7.1
Starch	21.2	27.9	27.6
Ash	7.8	8.1	8.4
Ca	0.84	0.82	0.83
Total P	0.41	0.40	0.41

NE _L ⁴ (Mcal/kg of DM)	1.72	1.78	1.80
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¹GTMR = ground total mixed ration; PTMR = pelleted total mixed ration.

²Ca-LCFA = calcium soap of long-chain fatty acids.

³Contained (per kg): 460 g dried corn distillers grains with solubles; 360 g of limestone (38 % of Ca); 8.5 g of magnesium oxide (54% Mg); 32 g of P; 10 g of Fe; 3 g of Cu; 0.7 g of Mn; 1.3 g of Zn; 13 mg of Co; 33 mg of I; 600 mg of Se; 1700,00 IU of vitamin A; 330,000 IU of vitamin D; and 1,100 IU of vitamin E. Purchased from Portal Agri-Industries Co., Ltd., Nanjing, Jiangsu, China.

⁴Calculated using NE_L values of feedstuffs from NRC (2001).

Table 2 Effects of feeding ground versus pelleted total mixed ration on feed intake, feed digestion, and rumen fermentation of dairy cows.

Index ²	Diets ¹		SEM	P value
	GTMR	PTMR		
Dry matter intake (kg/d)	29.30 ^b	30.06 ^a	0.12	<0.001
Average daily gain (kg/d)	0.48 ^b	0.73 ^a	0.02	<0.001

Feed conversion ratio (DMI/ADG)	62.20 ^a	41.20 ^b	2.31	<0.001
Apparent digestibility (%)				
Dry matter	65.67 ^b	73.37 ^a	1.81	0.013
Crude protein	53.46 ^b	62.18 ^a	1.93	0.004
Neutral detergent fiber	53.00 ^a	42.76 ^b	1.68	0.008
Acid detergent fiber	41.55	34.82	2.17	0.063
Ether extract	64.42	66.13	1.30	0.371
Ash	76.33	76.52	1.24	0.914
Starch	83.61 ^b	90.54 ^a	1.27	0.003
Rumen fermentation parameters				
pH	6.48 ^a	6.10 ^b	0.06	<0.001
Ammonia nitrogen (mmol/L)	12.93 ^a	11.03 ^b	0.47	0.016
Total volatile fatty acid (mmol/L)	87.11	86.76	1.54	0.875
Volatile fatty acid profile (mol/100 mol)				
Acetate	65.17 ^a	59.90 ^b	0.70	<0.001
Propionate	23.07 ^b	27.86 ^a	0.58	<0.001
Acetate : Propionate	2.85 ^a	2.15 ^b	0.10	<0.001
Butyrate	7.02	7.27	0.36	0.626
Isobutyrate	1.71	1.68	0.09	0.823
Valerate	1.34	1.44	0.10	0.491
Isovaleric acid	1.70	1.84	0.11	0.405

Means within a row with different superscript letters (a, b) are different (P < 0.05).

¹GTMR, ground total mixed ration; PTMR, pelleted total mixed ration.

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Table 3 Effects of feeding ground versus pelleted total mixed ration on blood metabolites of dairy cows.

Index	Diets ¹			Feeding day					P value		
	GTMR	PTMR	SEM	0	14	28	42	SEM	Diet	Day	Diet * Day
Total protein (g/L)	74.78	70.16	2.32	75.98	67.82	73.87	72.23	3.28	0.167	0.356	0.639
Albumin (g/L)	28.57	30.14	0.72	29.61	27.62	30.98	29.20	1.02	0.132	0.156	0.779
Globulin (g/L)	46.22	40.02	2.40	46.37	40.20	42.88	43.03	3.39	0.075	0.648	0.647
Triglyceride (mmol/L)	0.17	0.16	0.01	0.17	0.16	0.17	0.16	0.01	0.191	0.582	0.510
Total cholesterol (mmol/L)	5.00	5.46	0.24	5.71	4.86	5.05	5.31	0.34	0.181	0.326	0.990
Urea nitrogen (mmol/L)	5.10	5.51	0.19	4.91 ^b	4.99 ^b	5.23 ^{ab}	6.10 ^a	0.26	0.133	0.010	0.631
Glucose (mmol/L)	3.35	3.25	0.09	3.24 ^{ab}	3.01 ^b	3.47 ^{ab}	3.49 ^a	0.13	0.411	0.032	0.775
Alkaline phosphatase (IU/L)	32.71	35.25	3.17	32.58	30.92	36.42	36.00	4.48	0.574	0.786	0.742

33 Means within a row with different superscript letters (a, b) are different ($P < 0.05$).34 ¹GTMR = ground total mixed ration; PTMR = pelleted total mixed ration.

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40 **Table 4** Effects of feeding ground versus pelleted total mixed ration on milk yield and compositions of dairy cows.

Index ²	Diets ¹	SEM	Feeding day	SEM	P value
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	GTMR	PTMR		0	7	14	21	28	35	42		Diet	Day	Diet * Day
Milk yield (kg/d)	36.41 ^a	35.12 ^b	0.39	38.47 ^a	37.16 ^{ab}	37.11 ^{ab}	34.83 ^{bc}	35.04 ^{bc}	33.24 ^c	34.49 ^{bc}	0.72	0.021	<0.001	0.326
4% FCM (kg/d)	32.63 ^a	30.91 ^b	0.37	33.57	32.48	32.64	31.15	31.60	29.80	31.14	0.70	0.002	0.009	0.039
Milk fat (%)	3.32 ^a	3.20 ^b	0.03	3.15	3.16	3.19	3.29	3.35	3.31	3.35	0.06	0.019	0.107	0.127
Milk fat (kg/d)	1.20 ^a	1.13 ^b	0.02	1.21	1.18	1.19	1.15	1.17	1.10	1.16	0.03	0.001	0.287	0.012
Milk protein (%)	3.16 ^a	3.38 ^b	0.02	3.20	3.20	3.12	3.32	3.32	3.36	3.36	0.05	<0.001	0.001	0.002
Milk protein (kg/d)	1.15	1.18	0.01	1.24	1.23	1.19	1.16	1.16	1.11	1.15	0.03	0.122	0.114	0.553
Milk SNF (%)	8.55	8.70	0.07	8.43	8.75	8.38	8.82	8.72	8.57	8.74	0.13	0.139	0.131	0.654
Milk SNF (kg/d)	3.11	3.05	0.04	3.24 ^a	3.25 ^a	3.11 ^{ab}	3.08 ^{ab}	3.05 ^{ab}	2.84 ^b	3.01 ^{ab}	0.07	0.246	0.001	0.498
Milk lactose (%)	4.73	4.79	0.04	4.75	4.80	4.55	4.84	4.82	4.77	4.79	0.07	0.292	0.127	0.758
Milk lactose (kg/d)	1.72	1.68	0.02	1.83 ^a	1.78 ^{ab}	1.69 ^{abc}	1.69 ^{abc}	1.68 ^{abc}	1.58 ^c	1.65 ^{bc}	0.04	0.154	0.002	0.685
Milk ash (%)	0.69	0.70	0.01	0.68	0.71	0.67	0.71	0.71	0.70	0.71	0.01	0.266	0.079	0.822
Milk ash (kg/d)	0.25	0.25	0.01	0.26 ^a	0.26 ^a	0.25 ^{ab}	0.25 ^{ab}	0.25 ^{ab}	0.23 ^b	0.24 ^{ab}	0.01	0.161	0.006	0.673

Means within a row with different superscript letters (a-c) are different (P < 0.05).

¹GTMR = ground total mixed ration; PTMR = pelleted total mixed ration.

²FCM, fat-corrected milk; SNF, solids not fat.

Table 5. Identified volatile compounds in milk of cows fed ground versus pelleted total mixed rations

No.	Volatile Compound	Chemical Formula	Molecular Weight	RT (min) ¹	Relative area to the peak area, % ²	
					GTMR ³	PTMR ³

1	Dodecanal	C12H24O	184	19.01	1.54	/
2	Heptadecanal	C17H34O	254	19.01	1.54	/
3	Octadecanal	C18H36O	268	19.01	1.54	/
4	Decanoic acid, octyl ester	C18H36O2	284	16.09	/	0.79
5	Decanoic acid, decyl ester	C20H40O2	312	16.09	/	0.79
6	di(Butoxyethyl)adipate	C18H34O6	346	16.09	/	0.79
7	Phthalic acid, hept-3-yl isobutyl ester	C19H28O4	320	18.55	1.07	/
8	1,2-Benzenedicarboxylic acid, butyl 2-methylhexyl ester	C16H22O4	278	19.48	0.62	/
9	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	C20H30O4	334	19.48	0.45	/
10	Phthalic acid, butyl isohexyl ester	C18H26O4	306	19.49	0.62	/
11	Undecanoic acid isopropyl ester	C18H35NO4	329	20.69	0.97	1.08
12	Diisooctyl phthalate	C24H38O4	390	25.51	2.37	1.69
13	Phthalic acid, di(2-propylpentyl) ester	C24H38O4	390	25.51	2.82	1.69
14	Hexanedioic acid, mono (2-ethylhexyl) ester	C14H26O4	258	23.53	/	1.08
15	Adipic acid, 2-ethylhexyl tetradecyl ester	C28H54O4	454	23.53	/	1.08
16	Hexanedioic acid, dioctyl ester	C22H42O4	370	23.54	12.05	10.47
17	2,3-Dihydroxybenzoic acid	C16H30O4	286	9.59	2.24	2.84
18	2,5-Dihydroxybenzoic acid	C16H30O4	286	9.59	1.81	1.00

19	2,6-Dihydroxybenzoic acid	C16H30O4	286	9.59	2.24	2.84
20	Phosphonoacetic Acid	C11H29O5P	272	12.06	12.13	8.28
21	9-Nonadecene	C19H38	266	17.69	7.52	1.84
22	10-Heneicosene	C21H42	294	17.69	5.64	14.77
23	5-Octadecene	C18H36	252	17.69	2.28	1.84
24	3-Octadecene	C18H36	252	17.69	1.36	/
25	3-Eicosene	C20H40	280	17.69	5.64	/
26	5-Eicosene	C20H40	280	17.69	5.25	14.77
27	9-Eicosene	C20H40	280	17.72	6.16	1.84
28	1-Hexadecanol	C16H34O	242	17.69	0.40	/

45 ¹RT = retention time

46 ² “/” means the concentration of compound was too low to be detected.

47 ³GTMR = ground total mixed ration; PTMR = pelleted total mixed ration.

Figure 1 Categories and relative peak areas for volatiles in milk. GTMR = ground total mixed ration; PTMR = pelleted total mixed ration. Means between both columns with different superscripts (a, b) are different ($P < 0.05$).

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