Indian Journal of Animal Sciences **90** (3): 439–445, March 2020/Article

In vitro evaluation of kinnow waste as substitute of cereal grains in the concentrate mixture and empty pea pods as that of berseem hay in total mixed ration for livestock

M P S BAKSHI*, J S HUNDAL and M WADHWA

Guru Angad Dev Veterinary and Animal Science University, Ludhiana, Punjab 141 004 India

Received: 30 August 2019; Accepted: 24 September 2019

ABSTRACT

This study was taken up to assess the extent to which kinnow mandarin (*Citrus reticulata*) waste (KW) could substitute cereal grains in the concentrate mixture and empty pea (*Pisum sativum*) pods (EPPs) as that of berseem (*Trifolium alexandrium*) hay in total mixed ration (TMR) for livestock. In the first experiment, sundried finely ground kinnow waste replaced barley grains on N basis at 0, 25, 50, 75 and 100% levels in concentrate mixtures. The comparable N-content indicated that the concentrate mixtures were iso-nitrogenous. The EE and cell wall constituents (CWC) increased linearly with the increase in level of KW. The replacement of barley with KW did not affect net gas production (NGP), true OM digestibility and ME availability up to 50% level of replacement. Total and individual volatile fatty acids (VFAs) and microbial biomass production improved up to 50% level of replacement but declined thereafter. In the second experiment, sundried finely ground EPPs replaced berseem hay on N basis at 0, 25, 50, 75 and 100% levels in TMR. The EE and CWCs content was not affected up to 50% level but thereafter EE content decreased, whereas CWCs content increased. The NGP, digestibility of true DM and OM, VFAs production, ME availability and microbial biomass synthesis improved up to 50% level of replacement of berseem hay with EPPs, thereafter it declined. The hydrogen recovery, hydrogen consumption and fermentation efficiency were not affected upto 50% level of EPPs but at higher levels these parameters were depressed. Therefore, KW could replace barley grains in concentrate mixture while EPPs could replace berseem hay in TMR up to 50% level without affecting nutrient utilization, VFA production, ME availability and microbial biomass production under *in vitro* conditions.

Keywords: Barley grains, Berseem hay, Empty pea pods, *In vitro*, Kinnow waste, Livestock, Nutrient utilization, Total mixed ration

The human population in Asia and Africa will increase by 41 and 47%, respectively in 2050. The general consensus is that global agriculture production has to be increased by 60–70% from the current levels to meet the increased food demand in 2050 (Silva 2018). Today, 55% of the world's population lives in urban areas, which is expected to increase to about 70% in 2050. According to the latest FAO projections, demand for meat in low and middle-income countries will increase by over 200% by 2050 and per capita meat consumption is likely to rise from 34 to 49 kg/annum (FAO 2018). Increased economic growth, income levels and urbanization of rural population in the developing countries are responsible for ever increasing demand of animal proteins especially meat and dairy products (Devi *et al*. 2014, Silva 2018). Consequently, it will result in increased feed demand.

There is shortage of feed in most of the Asian and African countries. In India, there is a shortage of 25, 159 and 117 million tonnes (mt) of concentrates, green forages and crop residues corresponding to a shortage of 32, 20 and 25% of the requirement (Ravi Kiran *et al*. 2012). Therefore, non-conventional, alternate feed resources which do not compete with human food will have to be explored. FAO (2014) estimated that at least one-third of the food produced in the world (estimated as 1.3 billion tonnes) is wasted every year. The losses and waste of horticultural commodities are the highest among all types of foods reaching up to 60% (Gustavsson *et al.* 2011). In this context, byproducts/wastes available from fast developing food processing industries like that of fruit and vegetables can play significant role in improving the availability of feed stuffs for livestock and poultry. The fruits and vegetable production in India increased from 65.6 and 128.45 mt to 97.36 and 184.4 mt, respectively from 2007–08 to 2017–18 resulting in 48.44 and 43.55% increase fruit and vegetables production (Anonymous 2018). Processing, distribution and consumption of fruit and vegetables resulted in about 25–30% wastage and their disposal in waste land and river *etc.* will lead to environmental pollution. An *in vitro* study was, therefore, taken up to assess the extent to which kinnow

*Corresponding author e-mail: bakshimps@yahoo.com

waste can substitute cereal grains in the concentrate mixture and empty pea pods as substitute of berseem hay in total mixed ration without affecting nutrient utilization, VFA production, ME availability and microbial biomass production.

MATERIALS AND METHODS

Kinnow waste and empty pea pods: Kinnow mandarin (*Citrus reticulata*) is a hybrid of two citrus cultivars—'King' (*Citrus nobilis*) × 'Willow Leaf' (*Citrus* × *deliciosa*). The mixture of peels, residual pulp and seeds left after the extraction of juice by the processing industry is called kinnow waste. Likewise after shelling peas (*Pisum sativum*) for human consumption, the leftover material is called empty pea pods or shells.

Chemical composition of feed stuffs: The finely ground samples of feed stuffs were analyzed for DM, CP and total ash (AOAC 2007), cellulose (Crampton and Maynard 1938) and other cell wall constituents like NDF, ADF and ADL (Van Soest *et al*. 1991). Hemicellulose was determined by difference in NDF and ADF.

In vitro *gas production studies:* The nutritional value of KW as substitute of barley grains in concentrate mixture and EPP as substitute of berseem hay in TMR were evaluated by *in vitro* gas production technique (IVGPT; Menke *et al*. 1979, Menke and Steingass 1988).

Three rumen fistulated male buffalo calves used as donor for rumen liquor were maintained on 2 kg conventional concentrate mixture (Maize 32, barley 20, soybean meal 15, groundnut extraction 15, rice bran 15, mineral mixture 2 and common salt 1% each), 2 kg green fodder and *ad lib.* wheat straw. The rumen contents were collected before feeding at 09:00 in a thermos flask flushed with CO_2 and maintained at 39°C. The rumen contents were blended for 2–3 min in a blender and strained through four-layers of muslin cloth. The solution containing 960 ml distilled water, 0.16 ml micro-mineral solution, 660 ml bicarbonate buffer, 330 ml macro-mineral solution and 1.6 ml resazurine (0.1%) were mixed in a Woulff flask (3 L) with magnetic stirrer in a water bath at 39°C. The mixture was continuously flushed with CO_2. Then strained rumen liquor (SRL) was added to the buffer media in the ratio of 1:2.

Finely ground KW-concentrate mixtures (375±5 mg) or EPP–TMRs (on DM basis) was incubated in triplicate in a water bath at 39°C for 24 h in 100 ml calibrated glass syringes (Haberle Labortechnik, Germany) with buffered rumen fluid. If the volume of gas in the syringe exceeded 70 ml after 8 h, the volume was recorded and the gas was expelled. After 24 h, the volume of gas produced in each syringe was recorded and the contents of syringes were transferred to spout-less beaker, boiled with neutral detergent solution for assessing the true OM and NDF digestibility.

Estimation of volatile fatty acids: After 24 h of incubation, a 5 ml aliquot of fluid from each syringe was mixed with 1 ml of 25% meta-phosphoric and kept for 1 h at ambient temperature. Thereafter, it was centrifuged at 5500 rpm for 10 min and clear supernatant was collected and stored at –20°C until analyzed. The VFAs were estimated using Netchrom 9 100 gas chromatograph equipped with glass column (packed with chromosorb 101) and flame ionization detector (Cottyn and Boucque 1968). Temperature of injection port, column and detector was set at 250, 175 and 270°C, respectively. The flow rate of carrier gas (N_2) through the column was 15 ml/min; and the flow rate of H_2 and air through FID was 30 and 300 ml/min, respectively. Sample (2 µl) was injected through the injection port using a Hamilton syringe (10 µl). Individual VFA's of the samples were identified on the basis of their retention time and their concentration (mmol) and calculated by comparing the retention time as well as the peak area of standards after deducting the corresponding blank values.

Hydrogen recovery, hydrogen consumed *via* CH_4/VFA (Demeyer 1991), VFA utilization index which represents non-glucogenic VFAs to glucogenic VFAs ratio (NGGR), and microbial biomass synthesis and methane produced during fermentation were calculated from VFA concentration (Widiawati and Thalib 2009). The energetic efficiency of rumen fermentation (E; Ørskov *et al*. 1968), efficiency of conversion of fermented hexose energy to VFA energy (E_1; Czerkawski 1986) and efficiency of conversion of fermented hexose energy to CH_4 energy (E_2; IAEA 1985) were calculated from the molar proportion of VFAs (Baran and Zitnan 2002).

The data were analyzed by one way ANOVA (Snedecor and Cochran 1994) by using SPSS (2009) version 16.0 and the means were tested for the significant difference by Tukey's-b test.

RESULTS AND DISCUSSION

Experiment 1: Kinnow waste constituted 50% of processed kinnows. The citrus waste contains 60–65% peel, 30–35% internal tissues and up to 10% seeds (Ashbell *et al.* 1988). Due to the high moisture and sugar contents and presence of mould and yeast, citrus pulp gets rapidly deteriorated (Wing 2003, Nam *et al.* 2009) and may cause environmental pollution. Therefore, it should be sun dried or should be ensiled to increase its shelf life. The nutrients in fruit juice waste mainly from sweet lime, without peels were conserved by mixing and ensiling with wheat or rice straw in 70: 30 ratio (Bakshi *et al.* 2007).

The chemical analysis revealed that the sundried ground KW contained 8.28% total ash, 91.72% OM, 13.30% CP, 7.10% EE, 30.0% NDF, 23.3% ADF and 20.5% cellulose. The proximate components and cell wall constituents of KW observed in the present study were on the higher side than those observed in citrus pulp (Nellemann *et al*. 2009, FAO 2013). Citrus pulp is a rich source of trace elements; however, their concentration is much below the maximum tolerance limit for ruminants (Wadhwa *et al.* 2015). Datt *et al*. (2008) reported 63.74% IVDMD and 7.67 MJ ME/kg DM in orange peels.

The total ash content increased ($P<0.01$) while OM content decreased ($P<0.01$) linearly with the increase in level of kinnow waste in the concentrate mixtures. The comparable CP content indicated that the concentrate mixtures were isonitrogenous (Table 1). The NDF, ADF and cellulose contents increased (P<0.01) linearly with the increase in level of kinnow waste. The increase in EE, NDF, ADF and cellulose with the increase in level of replacement was mainly because kinnow waste had higher concentrations of these constituents as compared to barley

Table 1. Effect of replacing barley grains with kinnow waste on N-basis on the chemical composition of concentrate mixture

Parameter	Level of kinnow waste replacing barley grains in concentrate mixture (%)					PSE	P value
	0	25	50	75	100		
Ingredient composition (%)							
Barley	50.0	37.5	25.0	12.5	-		
KW	-	12.5	25.0	37.5	50.0		
GNC	20.0	20.0	20.0	20.0	20.0		
SBM	11.0	11.0	10.0	10.0	10.0		
Rice bran	16.0	16.0	17.0	17.0	17.0		
MM	2.0	2.0	2.0	2.0	2.0		
NaCl	1.0	1.0	1.0	1.0	1.0		
Chemical composition (% DM basis)							
Ash	7.00^{a}	7.85^{b}	8.55^{c}	9.05^{d}	9.35^{e}	0.28	<0.001
OM	93.00^{e}	92.15^{d}	91.45^{c}	90.95^{b}	90.65^{a}	0.28	<0.001
CP	21.65	21.75	21.40	21.60	21.90	0.12	0.828
EE	4.00^{a}	4.45^{b}	4.95^{c}	6.00^{d}	6.60^{e}	0.32	<0.001
NDF	24.87^{a}	25.00^{a}	25.70^{ab}	26.7^{bc}	27.20^{c}	0.32	0.002
ADF	15.95^{a}	16.50^{ab}	17.20^{bc}	17.80^{c}	19.45^{d}	0.41	<0.001
HC	8.92	8.50	8.50	8.9	7.75	0.11	0.909
Cellulose	11.10^{a}	11.60^{ab}	11.69^{ab}	12.10^{b}	13.22^{c}	0.24	0.001
ADL	3.50	3.60	4.10	3.40	4.40	0.14	0.068

KW, Kinnow waste; GNC, Groundnut extraction; SBM, Soybean meal; MM, Mineral mixture.

Table 2. Effect of replacing barley with kinnow waste on N-basis on the net gas production, digestibility, ME availability and volatile fatty acid production from concentrate mixture

Parameter	Level of kinnow waste replacing barley grains in concentrate mixture, %					PSE	P value
	0	25	50	75	100		
NGP (ml/24 h/g DM)	203.11^{b}	201.77^{b}	201.77^{b}	193.77^{ab}	188.44^{a}	1.72	0.002
TOMD (%)	85.81	86.52	85.98	87.66	86.99	1.14	0.499
TDMD (%)	86.53	87.20	86.80	88.27	88.00	0.65	0.357
PF	1.85^{a}	1.85^{a}	1.84^{a}	1.94^{ab}	1.98^{b}	–	0.016
ME (MJ/kg DM)	8.31	8.35	8.50	8.38	8.26	–	0.421
Volatile fatty acids production (mM/dL)							
TVFA	4.15^{b}	4.44^{c}	5.63^{e}	4.03^{a}	4.75^{d}	0.19	<0.001
Acetate (A)	2.28^{a}	2.47^{c}	3.31^{e}	2.38^{b}	2.94^{d}	0.13	<0.001
Propionate (P)	1.33^{c}	1.39^{d}	1.66^{e}	1.14^{a}	1.29^{b}	0.06	<0.001
Isobutyrate	$0.031b^{c}$	0.029^{ab}	0.043^{d}	0.026^{a}	0.034^{c}	0.002	<0.001
Butyrate	0.360^{b}	0.392^{c}	0.441^{d}	0.334^{a}	0.334^{a}	0.014	<0.001
Isovalerate	0.084^{b}	0.09^{d}	0.103^{e}	0.080^{a}	0.088^{c}	0.003	<0.001
Valerate	0.071^{c}	0.075^{d}	0.084^{e}	0.064^{a}	0.068^{b}	0.002	<0.001
A:P	1.72^{a}	1.78^{b}	2.00^{c}	2.08^{d}	2.28^{e}	–	<0.001
Relative proportion (%)							
Acetate	54.96^{a}	55.56^{b}	58.68^{c}	59.11^{d}	61.84^{e}	1.33	<0.001
Propionate	31.91^{e}	31.24^{d}	29.42^{c}	28.39^{b}	27.12^{a}	1.43	<0.001
Isobutyrate	0.74^{b}	0.66^{a}	0.76^{b}	0.65^{a}	0.72^{ab}	0.07	0.009
Butyrate	8.66^{d}	8.83^{d}	7.83^{b}	8.28^{c}	7.03^{a}	0.13	<0.001
Isovalerate	2.02^{d}	2.04^{d}	1.82^{a}	1.97^{c}	1.86^{b}	0.22	<0.001
Valerate	1.70^{e}	1.68^{d}	1.49^{b}	1.59^{c}	1.43^{a}	–	<0.001

NGP, Net gas production; TOMD, True OM digestibility; TDMD, True DM digestibility; PF, Partitioning factor; ME, Metabolizable energy; TVFA, Total volatile fatty acids; PSE, Pooled standard error. ^{a,b}Figures with different superscripts in a row differ significantly.

grains. The hemicelluloses and acid detergent lignin (ADL) contents were comparable in all the concentrate mixtures.

The replacement of barley with kinnow waste did not affect net gas production up to 50% level of replacement (Table 2) but it declined (P<0.001) after 75% level in the concentrate mixtures. Likewise ME content increased numerically (P>0.05) up to 50% level of replacement of barley grains with kinnow waste. The digestibility of true OM and that of DM was not affected by the level of kinnow waste. Assis *et al*. (2004) also reported that dried citrus pulp could be used as a cereal substitute in concentrate diets because of its high OM digestibility and energy availability (2.76–2.9 Mcal ME/kg DM and 1.66–1.76 Mcal NE/kg DM) for lactating dairy cows. The ME availability is 85–90% that of maize and comparable to barley (NRC 2001). The replacement of barley grains with kinnow waste had comparable partitioning factor up to 75% level which improved (P<0.05) thereafter.

The total and individual VFAs production improved up to 50% level of replacement of barley grains with kinnow waste and it declined thereafter. The relative proportion of acetate increased (P<0.01) while that of propionate decreased (P<0.01) with the increase in graded level of kinnow waste in the concentrate mixture. Unlike cereals, its energy is not based on starch but on soluble carbohydrates and digestible fibre. Citrus pectins are easily and extensively degraded producing acetic acid which is less likely than lactic acid to cause a pH drop and result in acidosis (Gohl 1982). Due to its high fibre content, the long rumination of citrus pulp produces large quantities of saliva that has a buffering effect on rumen pH. Citrus pulp is, therefore, considered as a safer feed than cereals for animals fed high-concentrate,

Table 3. Effect of replacing barley with kinnow waste on N-basis on the fermentation efficiency and microbial biomass synthesis from concentrate mixture

Parameter	Level of kinnow waste replacing barley grains in concentrate mixture (%)					PSE	P value
	0	25	50	75	100		
HR (%)	48.39^{e}	47.55^{d}	44.42^{c}	43.45^{b}	40.93^{a}	1.43	<0.001
HC	0.205^{a}	0.207^{a}	0.241^{b}	0.248^{c}	0.292^{d}	–	<0.001
E (%)	79.22^{e}	78.93^{d}	77.91^{c}	77.54^{b}	76.73^{a}	–	<0.001
E_1 (%)	84.42^{d}	84.22^{c}	82.66^{b}	82.59^{b}	81.09^{a}	0.64	<0.001
E_2 (%)	13.68^{a}	14.00^{b}	14.71^{c}	15.17^{d}	15.63^{e}	–	<0.001
MBM (g/day)	106.08^{b}	113.63^{c}	143.63^{e}	102.65^{a}	120.58^{d}	4.85	<0.001

HR, Hydrogen recovery; HC, Hydrogen consumed via CH_4/VFA; E, Efficiency of rumen fermentation; E_1, Efficiency of fermented hexose energy to VFA energy; E_2, Efficiency of fermented hexose to methane; VFA UI, VFA utilization index; MBM, Microbial biomass. ^{ab}Means with different superscripts in a row differ significantly.

Table 4. Effect of replacing *Trifolium alexandrium* (Berseem) hay with empty pea pods on N-basis on the chemical composition of total mixed ration

Parameter	Level of empty pea pods replacing berseem hay in total mixed ration, %					PSE	P value
	0	25	50	75	100		
Ingredient composition (%)							
Berseem	50.0	37.5	25.0	12.5	-		
EPP	-	12.5	25.0	37.5	50.0		
Barley	25.0	24.0	23.0	22.0	21.0		
Groundnut extraction	10.0	10.0	10.0	10.0	10.0		
Soybean meal	5.0	6.0	7.0	8.0	9.0		
Rice bran	8.0	8.0	8.0	8.0	8.0		
Mineral mixture	1.0	1.0	1.0	1.0	1.0		
NaCl	1.0	1.0	1.0	1.0	1.0		
Chemical composition (% DM basis)							
Ash	9.55^{d}	9.15^{d}	8.50^{c}	8.05^{b}	7.6^{a}	0.24	<0.001
OM	90.45^{a}	90.85^{a}	91.50^{b}	91.95^{c}	92.40^{d}	0.24	<0.001
CP	18.30	18.10	18.80	18.30	18.05	0.12	0.303
EE	3.30^{b}	3.00^{b}	3.20^{b}	2.40^{a}	2.50^{a}	0.12	0.001
NDF	40.58^{a}	41.10^{a}	41.20^{a}	42.70^{b}	43.05^{b}	0.33	0.001
ADF	25.50	25.70	26.20	26.10	26.65	0.16	0.092
HC	15.08^{a}	15.40^{a}	15.00^{a}	16.60^{b}	16.40^{b}	0.23	0.003
Cellulose	16.30^{a}	16.10^{a}	16.30^{a}	17.50^{b}	17.96^{b}	0.26	0.004
ADL	4.50^{a}	4.70^{a}	5.35^{b}	4.90^{ab}	4.90^{ab}	0.10	0.021

low-roughage diets as is practiced in high yielding dairy cows (Ashbell *et al*. 1988). No specific trend was observed in the relative proportion of remaining VFAs.

The hydrogen recovery, fermentation efficiency and efficiency of conversion of fermented VFA energy to hexose energy declined linearly with the increase in level of kinnow waste in the concentrate mixture, however, hydrogen consumption and microbial biomass synthesis increased up to 50% level of replacement, and declined thereafter significantly (Table 2). Gravador *et al*. (2014) reported that feeding citrus pulp at more than 40% level in complete feed depressed palatability, impaired nutrient absorption and animal performance.

Experiment 2: Fresh EPPs constitute 50–55% of garden peas. Ajila *et al*. (2012) reported that EPP constitute only 40–50% of garden peas. The sundried ground EPPs contained 6.23% total ash, 93.77% OM, 18.75% CP, 1.13% EE, 39.40% NDF, 23.55% ADF and 18.7% cellulose. However, CP content in pea pods was higher than reported earlier (Khattab *et al.* 2000, Khan and Atreja 2001). Earlier studies revealed that EPPs contained higher cell wall constituents than the values obtained in the present study (Bakshi and Wadhwa 2013). The variation in EPPs yield and chemical composition could be due to different agronomic practices adapted in different regions. The EPPs are rich in total soluble sugars (35.8%), total phenolics (9.4%) and macro and microminerals (Bakshi and Wadhwa 2013). Pea pods were found to possess 66.20% IVDMD and 8.01 MJ ME/kg DM (Datt *et al.* 2008).

The total ash content decreased (P<0.01) while organic matter content increased (P<0.01) linearly with the increase in level of EPPs replacing berseem hay in the total mixed rations (Table 4). The comparable crude protein content indicated that the TMRs were isonitrogenous. The replacement of berseem hay with EPPs up to 50% level did not affect EE, NDF, cellulose and hemicelluloses content but beyond 50% level of replacement EE content decreased (P<0.01), whereas cell wall constituents content increased (P<0.01). However, there was no difference in the concentration of these constituents at 75 and 100% replacement level. The ADF content exhibited similar trend, but the differences were non-significant.

The NGP improved (P<0.05) up to 50% level of replacement of berseem hay with empty pea pods (Table 5), thereafter it declined (P>0.05). The digestibility of true DM and OM improved (P<0.01) up to 50% level of replacement of berseem hay with empty pea pods, thereafter it declined significantly. The higher NGP, digestibility of DM and true OM resulted in higher ME availability from TMRs at 50% level of replacement of berseem hay with empty pea pods the ME availability declined thereafter. The digestibility of DM and OM was comparable to *in vivo* values obtained in bucks fed fresh EPPs *ad lib.* (Wadhwa *et al.* 2006). The partitioning factor was comparable in all the TMRs. Earlier reports revealed that fresh EPPs supplemented with mineral mixture and common salt can

Table 5. Effect of replacing *Trifolium alexandrium* (Berseem) with empty pea pods on N basis on the net gas production, digestibility, ME availability and volatile fatty acid production from total mixed ration

Parameter	Level of empty pea pods replacing berseem hay in total mixed ration, %					PSE	P value
	0	25	50	75	100		
NGP (ml/24 h/g DM)	188.0^{a}	195.11^{ab}	199.11^{b}	194.22^{ab}	192.89^{ab}	1.20	0.025
TOMD, %	78.42^{b}	79.15^{bc}	80.39^{c}	77.73^{b}	75.86^{a}	0.81	0.002
TDMD, %	79.87^{bc}	80.53^{bc}	81.60^{c}	79.07^{b}	77.20^{a}	0.82	0.004
PF	1.45	1.89	1.85	1.91	1.91	0.10	0.609
ME (MJ/kg DM)	7.77^{a}	7.88^{ab}	8.03^{b}	7.70^{a}	7.70^{a}	–	0.020
Volatile fatty acids production (mM/dL)							
TVFA	4.54^{b}	4.91^{d}	5.23^{e}	4.21^{a}	4.81^{c}	0.11	<0.001
Acetate (A)	2.88^{b}	3.10^{d}	3.29^{e}	2.66^{a}	3.02^{c}	0.07	<0.001
Propionate (P)	1.11^{b}	1.18^{c}	1.26^{d}	0.99^{a}	1.16^{c}	0.03	<0.001
Isobutyrate	0.031^{a}	0.038^{c}	0.041^{d}	0.032^{a}	0.035^{b}	0.001	<0.001
Butyrate	0.391^{a}	0.446^{c}	0.476^{e}	0.40^{b}	0.454^{d}	0.011	<0.001
Isovalerate	0.074^{a}	0.086^{b}	0.093^{c}	0.073^{a}	0.087^{b}	0.003	<0.001
Valerate	0.056^{b}	0.065^{d}	0.070^{e}	0.055^{a}	0.063^{c}	0.002	<0.001
A:P	2.60^{a}	2.63^{a}	2.62^{a}	2.68^{b}	2.61^{a}	–	0.001
Relative proportion (%)							
Acetate	63.46^{d}	63.04^{bc}	62.94^{b}	63.12^{c}	62.70^{a}	–	<0.001
Propionate	24.40^{c}	24.00^{b}	24.05^{b}	23.58^{a}	24.04^{b}	–	<0.001
Isobutyrate	0.68^{a}	0.78^{bc}	0.79^{c}	0.77^{bc}	0.74^{b}	–	0.001
Butyrate	8.60^{a}	9.09^{b}	9.10^{b}	9.48^{c}	9.42^{c}	–	<0.001
Isovalerate	1.62^{a}	1.76^{c}	1.79^{d}	1.73^{b}	1.80^{e}	–	<0.001
Valerate	1.24^{a}	1.33^{c}	1.34^{c}	1.31^{b}	1.31^{b}	–	<0.001

NGP, Net gas production; TOMD, True OM digestibility; TDMD, True DM digestibility; PF, Partitioning factor; ME, Metabolizable energy; TVFA, Total volatile fatty acids. [a,b]Figures with different superscripts in a row differ significantly; PSE, Pooled standard error.

Table 6. Effect of replacing *Trifolium alexandrium* (Berseem) with empty pea pods on N basis on the fermentation efficiency and microbial biomass synthesis from total mixed ration

Parameter	Level of empty pea pods replacing berseem hay in total mixed ration (%)					PSE	P value
	0	25	50	75	100		
HR (%)	38.41^{b}	38.37^{b}	38.46^{bc}	38.12^{a}	38.63^{c}	0.03	0.002
HC	0.27^{c}	0.26^{b}	0.26^{b}	0.25^{a}	0.25^{a}	-	<0.001
E (%)	75.70^{b}	75.65^{b}	75.68^{b}	75.53^{a}	75.72^{b}	0.23	0.002
E_1 (%)	80.86^{a}	81.08^{b}	81.12^{bc}	81.16^{c}	81.31^{d}	2.01	<0.001
E_2 (%)	17.03^{a}	17.22^{b}	17.20^{b}	17.44^{c}	17.24^{b}	0.53	<0.001
MBM (g/day)	116.23^{b}	125.49^{d}	133.41^{e}	107.79^{a}	123.19^{c}	2.89	<0.001

HR, Hydrogen recovery; HC, Hydrogen consumed via CH_4/VFA; E, Efficiency of rumen fermentation; E_1, Efficiency of fermented hexose energy to VFA energy; E_2, Efficiency of fermented hexose to methane; VFA UI, VFA utilization index; MBM, Microbial biomass. [ab]Means with different superscripts in a row differ significantly.

be fed *ad lib.* to adult ruminants without affecting productive performance of animals (Bakshi *et al*. 2016). The total and individual VFAs production improved (P<0.01) up to 50% level of replacement of berseem hay with empty pea pods (Table 5), thereafter it declined (P<0.01). The relative proportion of acetate and propionate decreased (P<0.01) with the increase in level of empty pea pods in the TMRs. No specific trend was observed in the relative proportion of remaining VFAs.

The hydrogen recovery and fermentation efficiency were not affected by replacing berseem hay by EPPs up to 50%. But at higher levels of replacement (75 and 100%), these parameters were depressed significantly (P<0.01). The efficiency of conversion of fermented VFA energy to hexose energy (E_1) and efficiency of conversion of fermented VFA energy to methane energy (E_2) increased with the increase in level of empty pea pods in the TMRs (Table 6). The microbial biomass synthesis increased up to 50% level of replacement and it declined (P<0.01) thereafter. Earlier reports indicated that microbial protein synthesis was second highest in animals fed fresh pea pods *ad lib.* as compared to those fed other fresh vegetable wastes *ad lib.* (Wadhwa *et al.* 2006).

Kinnow waste could replace barley grains in concentrate mixture, while empty pea pods could replace berseem hay in TMR up to 50% level without affecting nutrient utilization, VFA production, ME availability and microbial biomass production under *in vitro* conditions. Both the wastes are excellent source of nutrients for the livestock.

REFERENCES

Ajila C M, Brar S K, Verma M, Tyagi R D, Godbout S and Valero J R. 2012. Bio-processing of agro-byproducts to animal feed. *Critical Reviews in Biotechnology* **32**: 382–400.

Anonymous. 2018. Horticultural Statistics at a Glance 2018. Horticulture Statistics Division, Department of Agriculture, Cooperation and Farmers' Welfare, Ministry of Agriculture and Farmers' Welfare, Government of India. pp. 458.

AOAC. 2007. *Official Methods of Analysis*. 7th edn. Association of Official Analytical Chemists, Gaithersburg, Maryland, USA.

Ashbell G, Weinberg Z G and Arieli A. 1988. Effect of blanching on loss reduction in orange peel storage. *Journal Science and Food Agriculture* **45**: 195–201.

Assis A J, Campos J M S, Filho S, Queiroz A C, Lana R, Euclydes R F, Neto J M, Magalhaes A L R and Mendonca S. 2004. Citrus pulp in diets for milking cows. 1. Intake of nutrients, milk production and composition. *Revista Brasileira de Zootecnia* **33**: 242–50.

Bakshi M P S and Wadhwa M. 2013. Nutritional evaluation of cannery and fruit wastes as livestock feed. *Indian Journal of Animal Sciences* **83**: 84–89.

Bakshi M P S, Wadhwa M, Kaushal S and Ameir A A. 2007. *In vitro* evaluation of ensiled fruit and vegetable wastes. *Indian Journal of Animal Nutrition* **24**: 12–15.

Bakshi M P S, Wadhwa M and Makkar H P S. 2016. Wastes to worth: vegetable wastes and by-products as animal feed. *Commonwealth Agriculture Bureau Reviews* **11**: 012.

Baran M and Zitnan R. 2002. Effect of monensin sodium on fermentation efficiency in sheep rumen. *Archiv fur Tierzucht* **45**: 181–85.

Cottyn B G and Boucque C V. 1968. Rapid method for the gas chromatographic determination of volatile fatty acids in rumen fluid. *Journal of Agricultural and Food Chemistry* **16**: 105–07.

Crampton E W and Maynard L A. 1938. The release of cellulose and lignin content to the nutritive value of animal feeds. *Journal of Nutrition* **15**: 383–95.

Czerkawski J W. 1986. *An Introduction to Rumen Studies*. Pergamon Press, Oxford, 221–222.

Datt C, Aruna Chhabra, Singh N P and Bujarbaruah K M. 2008. Nutritional characteristics of horticultural crop residues as ruminant feeds in Tripura. *Indian Journal of Animal Sciences* **78**: 312–16.

Demeyer D I. 1991. Quantitative aspects of microbial metabolism in the rumen and hindgut. (Ed.) Jouany J.P. *Rumen Microbial Metabolism and Ruminant Digestion.* INRA Editions, Paris (France), pp. 217–37.

Devi S M, Balachandar V, Lee S I and Kim I H. 2014. An outline of meat consumption in the Indian population- A pilot review. *Korean Journal of Food Science and Animal Resources* **34**: 507–15.

FAO. 2013. Food Outlook. FAO, Rome. Available from http://www.fao.org/docrep/018/ al999e/al999e.pdf.

FAO. 2014. Definitional Framework of Food Losses and Waste. FAO Rome, Italy.

FAO. 2018. Shaping the future of livestock sustainably, responsibly, efficiently. The 10th Global Forum for Food and Agriculture (GFFA) Berlin, 18–20 January, 2018.

Gohl B. 1982. Les aliments du betail sous les tropiques. *FAO, Division de Production et Santé Animale*, Roma, Italy.

Gravador R S, Jongberg S, Andersen M L, Luciano G, Priolo A and Lund M N. 2014. Dietary citrus pulp improves protein stability in lamb meat stored under aerobic conditions. *Meat Science* **97**: 231–36.

Gustavsson J, Cederberg C, Sonesson U, Otterdijk R and Meybeck A. 2011. Global food losses and food waste: Extent causes and prevention. *International Congress Save Food!at Interpack,* Dusseldorf, Germany.

IAEA. 1985. Laboratory Training Manual on the Use of Nuclear Techniques in Animal Nutrition. Technical Reports Series No. 248, International Atomic Energy Agency, Vienna.

Khan M L and Atreja P P. 2001. Chemical composition of vegetable residues from agro processing plants. *Proceeding of the Animal Nutrition Conference* held from 4–7 October at NDRI, Karnal.

Khattab H M, Abo El-Nor S A H, El-Sayed H M, Sadda Y M and El-Shaffy O H A. 2000. Utilization of some agro-industrial byproducts combination in ration of lactating goats. *Egyptian Journal of Animal Sciences* **28**: 85–96.

Menke K H and Steingass H. 1988. Estimation of the energetic feed value obtained by chemical analysis and *in vitro* gas production using rumen fluid. *Animal Research and Development* **28**: 755.

Menke K H, Rabb L, Salewski A, Steingass H, Fritz D and Schneider W. 1979. The estimation of the digestibility and ME content of ruminant feedstuffs from the gas production when they are incubated with rumen liquor *in vitro*. *Journal of Agricultural Science Cambridge* **93**: 217.

Nam I S, Garnsworthy P C and Ahn J H. 2009. Effects of freeze-dried citrus peel on feed preservation, aflatoxin contamination and *in vitro* ruminal fermentation. *Asian Australasian Journal of Animal Science* **22**: 674–80.

Nellemann C, MacDevette M, Manders T, Eickhout B, Svihus B, Prins A G and Kaltenborn B P. 2009. The environmental food crisis—The environment's role in averting future food crises. A UNEP rapid response assessment. United Nations Environment Programme, GRID-Arendal.

NRC. 2001. *Nutrient Requirements of Dairy Cattle*. 7th revised edn. National Research Council, National Academy of Sciences, Washington, DC, USA.

Ørskov E R, Flatt W P and Moe P W. 1968. Fermentation balance approach to estimated extent of fermentation and efficiency of volatile fatty acid formation in ruminants. *Journal of Dairy Science* **51**: 1429–35.

Ravi Kiran G, Suresh K P, Sampath K T, Giridhar K and Anandan S. 2012. *Modeling and forecasting livestock and fish feed resources: Requirements and Availability in India.* National Institute of Animal Nutrition and Physiology, Bengaluru, Karnataka, India.

Silva G. 2018. Feeding the world in 2050 and beyond – Part 1: Productivity challenges. Michigan State University Extension. www.canr.msu.edu./people/George_silva

Snedecor G W and Cochran W G. 1994. *Statistical Methods*. 7th edn. Oxford and IBH Publications, New Delhi, India.

SPSS. 2009. *Statistical Packages for Social Sciences.* Version 16. SPSS Inc., Illinois, USA.

Van Soest P J, Robertson J B and Lewis B A. 1991. Methods for dietary fibre, neutral detergent fibre, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**: 3583–97.

Wadhwa M, Bakshi M P S and Makkar H P S. 2015. Waste to worth: fruit wastes and by-products as animal feed. *Commonwealth Agriculture Bureau Reviews* **10**: 031.

Wadhwa M, Kaushal S and Bakshi M P S. 2006. Nutritive evaluation of vegetable wastes as complete feed for goat bucks. *Small Ruminant Research* **64**: 279–84.

Widiawati Y and Thalib A. 2009. Comparison of fermentation kinetics (*in vitro*) of grass and shrub legume leaves: The pattern of VFA concentration, estimated CH_4 and microbial biomass production. *Indonesian Journal of Agriculture* **2**: 21–27.

Wing J M. 2003. *Citrus Feedstuffs for Dairy Cattle*. University of Florida, IFAS. p. 829.