Profiling of in vitro rumen digestibility, fermentation parameters and fatty acid biohydrogenation of palm kernel cake-based diet supplemented with corn

Osama Anwer Saeed (osama_anwr85@uoanbar.edu.iq)
University of Anbar https://orcid.org/0000-0003-2706-1332
Umar M. Sani
Taraba State University
Awis Q. Sazili
University Putra Malaysia: Universiti Putra Malaysia
Henny Akit
University Putra Malaysia: Universiti Putra Malaysia
Abdul R. Alimon
Universitas Gadjah Mada
Anjas A. Samsudin
University Putra Malaysia: Universiti Putra Malaysia

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Abstract

Corn supplementation can enhance the function of rumen and mitigate methane production. Thus, this study aimed to evaluate *in vitro* rumen digestibility, fermentation parameters and fatty acid biohydrogenation of palm kernel cake-based (PKC) diet substituted with different levels of corn. Corn was substitution into PKC basal diet at the levels; T1= (0% corn + 75.3% PKC), T2= (5% corn + 70.3% PKC) and T3= (10% corn + 65.3% PKC) of the diet. Rumen liquor was obtained from four fistulated Dorper sheep and incubated with 200 mg of each treatment for 24hrs and 72hrs. Net gas production, fermentation kinetics, *in vitro* organic matter digestibility (IVOMD), *in vitro* dry matter digestibility (IVDMD), volatile fatty acids (VFA), rumen microbial population and fatty acid biohydrogenation were determined. The results of the *in vitro* study showed that production of gas increased from 0 hr until 9 hrs with T2 having the highest gas production during this phase. After 48 hrs, the gas production began to decrease gradually with increase in incubation time. No significant differences were observed in the IVDMD, IVOMD, NH_3-N, pH and VFA at 72 hrs. However, higher significant methane gas ($CH_{4)}$ production was observed in T3 when compared with T1 and T2. Microbial population did not differ significantly between treatment groups for total bacteria, *F. succinogenes* and *R. flavefaciens*. The rates of biohydrogenation were not affected by corn substitution although a significant difference was observed in that of C18:1n9. In conclusion, corn substitution maintained fermentation characteristics with increasing of unsaturated fatty acids.

Introduction

The high prices of grains have forced farmers to seek alternative sources to feed their livestock. South East Asian countries such Malaysia and Indonesia are blessed with abundant oil palm resources which generated a huge volume of oil palm by-product like palm kernel cake (PKC). The amounts of nutrients in the PKC are considered to have moderate crude protein content and its used as feed for livestock's feeding (Alimon 2004). The protein content of PKC is between 16–18%, which is considered to be moderate digestible crude protein (Alimon 2004). Chanjula et al. (2010) reported the inclusion of up to 30% PKC in the diets of goats on a digestibility trial that lasted 21 days with no adverse effects on rumen fermentation characteristics, nutrient utilization, and microbial populations. However, corn has been a good source energy in ruminants' diet. Recently, corn supplementation has become a common practice to increase the energy density of diets for high producing ruminants (Saeed et al. 2019a; Saeed et al. 2019b; Xu et al. 2019). In this regard, partial substitution of corn into PKC based diet could increase the rate of fermentation and rumen contractions. There are limited information on the usage of PKC as a major component of high-concentrate diets in addition to corn in an *in vitro* fermentation technique of Dorper sheep. Therefore, the objective of this study was evaluated the *in vitro* rumen digestibility and fermentation parameters of a PKC-based diet substituted with different levels of corn.

Materials And Methods

Donor animals

Four Dorper sheep with body weight of 28 ± 0.54 kg (mean ± standard deviation) and fitted with permanent rumen fistula were used in this study. The animals were fed on roughages and a commercial concentrate diet and kept individually in a cage. The fistulated sheep were fed two times a day, water and mineral blocks were provided *ad-libitum*. Equal volumes of rumen liquor were obtained from the four fistulated Dorper sheep prior to morning feed and strained through four layers of cheesecloth into a thermos flask which was flushed with CO_2 and immediately transported to the laboratory for analysis.

Treatment diets

Three dietary treatments T1: control diet (0% corn + 75.3% PKC), T2 diet containing (5% corn + 70.3% PKC), and T3 diet (10% corn + 65.3% PKC) were formulated. The three diets were approximately isonitrogenous. Minerals premix are excluded from the diets due to the high content of essential minerals in PKC so as to reduce the amount of Cu in the diets. The chemical composition and ingredients of experimental diets are presented in Table 1.

Chemical analysis

The dry matter, crude protein, ether extract, and ash content of the treatments were determined according to (AOAC 1990). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to the method of Van Soest (1994).

Sampling

The experiment was conducted in three runs. At the end of the trials, the *in vitro* samples were pooled based on time and treatment (0, 24, and 72 h of incubation). Net gas production was examined at 0, 3, 6, 9, 12, 24, 48, and 72 h. The pH of the rumen buffer were measured at 0 h and after 72 h of incubation using pH meter (Mettler-Toledo, Ltd, Leicester, UK) while substrate was collected from syringes after 0, 24, and 72 h of incubation and divided into two falcon tubes. Two to three drops of 10% H_2SO_4 were added into one tube which was used for NH_3-N analysis while the other tube was used for fatty acid and biohydrogenation analysis and microbial population. The substrate in both tubes were kept at -20^{o}C till analysis.

Kinetic fermentation

The computed gas production was according to the model of Ørskov and McDonald (1979).

$V= a+ b (1-e^{-ct})$ using Neway software

Where,

V = volume of gas formed at time t.

a = amount of gas produced from soluble fraction.

b = volume of gas produced at an insoluble fraction.

c = gas production rate constant for the insoluble fraction.

T = incubation time.

Rumen fermentation assessment

In vitro dry matter digestibility was ascertained after 72 h incubation according to (Menke and Steingass 1988). The blank and sample contained in the glass syringes were poured into a pre-weighed sintered glass, the syringes properly rinsed with distilled water to remove all residues, and the water evacuated completely from the sintered glass with the aid of a vacuum pump. Then the sintered glass and its contents were dried for 24 h in an airtight oven at 105^{o}C. Ammonia nitrogen (NH_3-N) was determined in accordance to Parsons et al. (1984). The determination of volatile fatty acid (VFA) was done using a gas chromatograph (Hewlett Packard 6890 GC system) according to the procedure of Cottyn and Boucque (1968). Methane generated during *in vitro* rumen fermentation of the feeds in the syringes was approximated utilizing the equation based on VFA proportion according to (Widiawati and Thalib 2012).

$CH_4 = 0.5 \times (A) + 0.5(B) - 0.25 \times (P)$

Where,

CH_4= amount (mmol) of methane produced

(A)= concentration (mmol) of acetate

(B) = concentration (mmol) of butyrate

(P) = concentration (mmol) of propionate

Bacteria quantification

The extraction of the total bacterial DNA was done using the QIAamp® DNA Mini stool kit (Qiagen, Hilden, GmbH) in accordance with manufacturer's instruction with some changes. Species-specific quantitative real-time PCR was carried out using CFX96 Touch Real-Time PCR Detection System (BioRad, USA) with an optical grade plate of SYBR Green mix detection. The microbial populations such as total bacteria, cellulolytic bacteria, methanogenic archaea and total protozoa were determined by quantitative real-time PCR according to (Saeed et al. 2018).

Fatty acid analysis

The fatty acid composition of the rumen substrate was incubated for 0, 24, and 72 h. The rumen fluid was removed from the freezer and allowed to thaw at room temperature for about 45 minutes. The total

lipid extraction method described by Folch et al. (1957) was used for extraction of fatty acids from the feed and rumen fluid samples.

Apparent biohydrogenation of oleic (C18:1n-9c), linoleic (C18:2n-6c), and linolenic (C18:3n-3) acid was determined based on the difference in the concentration of fatty acids between 0 h and 24 h *in vitro* incubation using the following formula (Adeyemi et al. 2015):

Apparent biohydrogenation (%) = $[100 \times [(CFA)_i\text{-}(CFA)_f / (CFA)_l]$

Where,

$(CFA)_l$ = % concentration of unsaturated fatty acid at 0 h incubation

$(CFA)_f$ = % concentration of unsaturated fatty acid at 24 h incubation

Statistical analysis

The chemical composition and secondary compound metabolism of forage samples were analyzed using a simple mean, while other data were subjected to one-way analysis of variance (ANOVA) of SAS, (9.4). Means were separated using Turkey.

Results

In vitro gas production and fermentations of rumen liquor

There were no significant differences ($P>0.05$) in *in vitro* kinetic fermentation (Table 2). Figure 1 shows profile of the cumulated gas production of T1, T2, and T3 following incubation in the buffered rumen fluid of sheep. The outcome indicated that gas production increased gradually and reached its peak at an incubation time 9 h, T2 recorded the highest gas production when compared to other treatment groups. However, after 48 h of incubation gas production began to decline gradually with passing of time. No significant differences ($P>0.05$) were observed in IVDMD, IVOMD, and NH_3-N and pH at 72 h. However, significant effect of CH_4 was observed with T3 showing the highest CH_4 concentration when compared with T1 at 2.81, 2.71, and 3.72 (mmol/L of gas) respectively (Table 3).

Volatile fatty acid

Table 4 showed the quantum of each VFA product (acetate, propionate, butyrate, isopronate and isobutyrate) after *in vitro* incubation. No significant difference was observed on overall VFA at 0 and 72 hrs and mean overall of incubation among the treatment groups. However, at 72 hrs of incubation only propionate, isopropionate and butyrate showed significant difference ($P<0.05$) when corn was used as the energy source. The results showed higher propionate and butyrate with low-level isopropionate recorded in T3 at 72 h of incubation as compared to T1 and T2. Also, no significant differences were observed among treatments in isobutyrate, and C2: C3 in the incubation period.

The Rumen microbial profile

The effect of treatment on total bacteria population in the rumen liquor measured at different hours of *in vitro* experiment is presented in Table 5. The total bacteria in the rumen liquor concentration was significantly higher in the corn substituted group (P<0.01) at 0 hr of the trial. However, no significant differences were observed on the total bacteria population after 24 hrs of incubation. The population of cellulolytic bacteria (*Fibrobacter succinogenes, Ruminococcus albus*, and *Ruminococcus flavefaciens*) was not significantly different among the dietary treatments. Furthermore, the *F. succinogenes* showed a reduction pattern between 0-24 hrs for T2 and T3 than T1 which start to increase gradually as the trial progressed. However, the population of *R. albus* and *R. flavefaciens* remained unchanged in all treatments during the entire period of the experiment. In this study, the methanogenic archaea population gradually increased in the rumen liquor from 0 h to 24 hrs. At 24 h of incubation, the total methanogenic archaea increased (P<0.001) in the rumen liquor as much as 5.56 and 5.12×10^9/ml in T1 and T3, respectively while T2 remained unchanged with 4.12×10^9/ml. Moreover, the overall mean of T1 caused an increase in the average of rumen methanogenic archaea population (P<0.001) compared to T2 and T3 (5.27, 4.02, and 4.43 10^9/ml respectively). The effect of different levels of corn as substitute into PKC urea-treated rice straw based diet on the rumen protozoa population in the rumen liquor was quantified. The mean concentration of protozoa in ruminal fluid was significantly (P<0.001) higher at 24 h in T2 than in the other treatments. The average number of protozoa were significantly (P<0.001) affected by 3.88, 5.39, and 4.12 10^5/ml respectively. The protozoa population in the rumen liquor slightly increased at 24 h in all treatments except for T3 where it has recorded the lowest population compared with the rest of the time in the treatments.

Fatty acid composition of rumen liquor

Table 6 presents the fatty acid composition of rumen inoculum after 24 h *in vitro* incubation. The corn levels did not affect (P>0.05) on C12:0, C14:0, C15:0), 15:1, C16:0, C18:0, C18:1n9c, linoleic (C18:2n6c) linolenic (C18:3n-3), and docosahexaenoic (C22:6n-3). There were significant differences (P<0.001) in concentrations of C16:1n-9 and C20:5n-3 while the total saturated fatty acids, total unsaturated fatty acids, and C22: n5-6 were not significant (P>0.05) among treatments. The end product of rumen biohydrogenation is C18:0, and from the results it was observed that its concentration was lower in T2 than T3. Similarly, the rates of biohydrogenation of C18:2n-6c and C18:3n-3 were not affected by corn substitution although a significant difference (P<0.05) was observed in that of C18:1n9.

Table 7 shows the fatty acid profile of rumen liquor after 72 h of incubation differs at the level of corn to palm kernel cake ratio. The addition of corn increased significantly (P<0.05) the proportion of medium chain FA (C12:0, C15:1, C16:0). It also significantly (P<0.01) increased the percentage of C18:1*trans*-11, C18:1n-9 but reduced the percentage (P<0.05) of C18:0, C20:4n-6 and C22:5n-3. The ability of corn to grow Σ *Trans* FA was more (P<0.05) noticeable in high concentrate substrates. However, small substitutions of substrates had significantly (P<0.05) higher biohydrogenation intermediates at T1. Apparent biohydrogenation of C18:2n-6 and C18:3n-3 was not as affected as the level of corn substituted

increased in the substrate. Corn substitution reduced (P<0.001) the apparent biohydrogenation of C18:1n-9 in T2 and T3 compared with T1.

Discussion

In vitro gas production and fermentations of rumen liquor

Amount of gas produced during *in vitro* incubation could reflect the degree of degradability and fermentation of a substrate. Substitution of corn had no effect on, gas production kinetics, IVDMD, IVOMD, pH, and NH_3-N. The present finding is consistent with Chanjula et al. (2010) who reported that an inclusion of up to 30% PKC in ruminants diet did not adversely affects rumen fermentation characteristics and microbial populations. There was no significant difference in gas production for the soluble fraction (a), insoluble fraction (b), potential gas production (a + b), and gas production rate constant with the insoluble fraction (c) upon corn substitution for all the three treatments in this study. This was probably due to the inability of a microorganism to utilize the nutrients bound to the structural components of PKC under Maillard reaction that occurred during processing of oil extraction (Sundu and Dingle 2003). Cumulative gas production after 24 h of incubation increased when 5% of corn was included in the diet (T2) and a tendency to a curvilinear pattern is noted when the maximum result was approached. The increase in cumulative gas production is due to the higher initial fermentation rate of the corn treatments. Ruminal NH_3-N and pH at 0 and 72 h incubation were not altered by diets containing corn with PKC urea-treated rice straw based diets, ranging from 31-36 mg/dL and pH 6.69 - 6.79 at 72 h. The level of ruminal NH_3-N exceeded 5-8 mg/dL in all treatment groups, which is the optimal level of NH_3-N for microbial protein synthesis (Mamuad et al. 2019; Satter and Slyter 1974), also all treatment pH means were within the normal range with the values showing relative stability at 6.22-6.53, which is the optimal level for microbial digestion of fiber and protein 6.0-7.0 (Chanjula et al. 2010; Chen et al. 2016; Van Soest 1994). Higher rumen NH_3-N concentration of 36.79 mg/dL was observed at T1 while 35.69 mg/dL was recorded at T2. This is a relatively greater concentration of rumen NH_3-N and agreed with Satter and Slyter (1974) who reported 5 mg/dL as minimum level of rumen NH_3-N for optimum microbial protein synthesis. The higher level of NH_3-N observed in this study may probably be due to the high proportion of non-protein nitrogen (NPN) in the rice straw. The declined of NH_3-N concentration might be due to more efficient N-utilization by rumen microbes when fermentable energy was available. As shown in the present study, dietary components such as NDF and ADF also contribute to the difference in CH_4 production. High levels of NDF raise CH_4 production by moving the short-chain fatty acid fraction towards acetate which is responsible for producing more hydrogen. The type of carbohydrate present in the diet is thought to dictate CH_4 production via changes in the ruminal microbial (Johnson and Johnson 1995).

Voliatile fatty acid

Fermentation in the rumen produces volatile fatty acids, such as acetate, propionate, and butyrate acids, which can be metabolized by the animal. In this study, the means of total VFA, acetate, propionate, and butyrate concentrations in the rumen were unaffected by dietary treatments but in terms of the numbers and the overall level of VFA was a little lower in T1 when compare with other treatments, most likely because of the low apparent digestibility. In present study, there was a continuous decrease in propionate and butyrate production but no difference in acetate after 72 h which may be due to the present of rice straw that is high in fiber in the diet. Treatment groups substituted with 5% and 10% corn (T2 and T3) showed a significant increase in propionate at 72 hrs. Generally, in ruminants, rapidly fermentable substrates have a relatively higher propionate acid production. On the other hand, slowly fermentable and cellulose-rich substrates will have high acetate acid-directed fermentation products. High concentrations of soluble carbohydrate promotes propionate production in the rumen, lower ruminal pH, and inhibits methanogen growth, thereby reducing CH_4 production per unit of fermented organic matter. This is in agreement with Atasoy et al. (2019) who reported that VFA produced in the rumen is affected by several factors such as pH, feed composition, and microbial species. Dias (2010), however, affirms that the high levels of PKC in the diet of goats probably reduces the digestibility of energy from PKC mainly because of the low content of starch. Butyrate production was significantly higher in T3 than the control at 72 h of incubation and this could be as a result of ruminal protozoa domination that produces butyrate acid as their end product of carbohydrate fermentation (Saeed et al. 2018; Williams and Coleman 1997). The acetate-propionate ratio was the same across dietary treatments in the present study as reported by (Dayani et al. 2007).

Rumen microbial profile

Several reports showed that dietary corn substitution affects total bacteria populations in rumen (Saeed et al. 2018). The total bacteria population in T2 and T3 demonstrates that it is significantly higher at 0 h but declined at 24 h. This may be due to the increased in the number of protozoa and their engulfing effect on the rumen bacteria. This is contrast to Abubakr et al. (2014) who reported that an increase in rumen bacteria population in goats fed PKC based diet could be due to the rapid multiplication after elimination of protozoa. The reason for this is not clear but it could be due to bacterial predation by rumen protozoa which is mainly reliant on the size and type of protozoa such as the holotrich protozoa which has much lower predatory activity than entodiniomorphids. Moreover, the mean of rumen total bacteria population in this *in vitro* trials had significantly reduced in T1, it is well established that fatty acids composition in substrate could be toxic to total bacteria (Williams and Coleman 1997) while long or short-term defaunation may reduce the rumen bacteria population. Methanogens archaea respond differently to different levels of corn at 24 h. In our experiment, the relative abundance of methanogens archaea slightly increased in response to corn in T3, was reported to be affected slightly (Lu et al. 2020). Methanogenesis frequently makes use of the hydrogen and CO_2 from the fermenting of carbohydrate as VFA are formed. By eliminating hydrogen from the ruminal environment as a last step of carbohydrate fermentation, methanogens permit the microorganisms active in fermentation to function at an optimal rate and to form the complete oxidation of substrates. With regard to this, it could be that increasing

methanogens archaea from the higher degradability of feed in T3 at 24 h that releases H_2 subsequently enhances the ability of methanogens archaea to grow. Johnson and Johnson (1995) stated that the digestion of cell wall fiber enhances CH_4 production by raising the quantum of acetate production and lowering the propionate produced. The rise in CH_4 emission is because of the fermenting of acetate which creates a methyl group for methanogenesis. The level of methanogens observed in T2 are an indication of low CH_4 emission since the hydrogen ion needed by methanogens to reduce CO_2 to CH_4 was drastically reduced (Bhatta et al. 2015). As a component of medium chain fatty acids, it can potentially hamper rumen methanogenesis and methanogens because of their toxic effect. Rumen protozoa are in control in the symbiotic transfers of H_2 with methanogens which is utilized to decrease CH_4 and produce over 25% of CH_4 in the rumen. Meanwhile, Machmüller et al. (2003) indicated that the relationship between protozoa and methanogens does not play a significant role in rumen methanogenesis. Changes in the proportion of the different methanogen species are due to a lower O_2 pressure and greater H_2 availability within the protozoal cells (Williams and Coleman 1997). Rumen protozoa were affected by treatments, at 0 and 24 h incubation and overall protozoal populations increased by the substitution of 5% corn as source of energy in the treatment (T2), perhaps as a result of higher level of corn in the diets which enhanced domination of protozoa on the rest of rumen microbial population. This finding is agreed with that of Abdullah and Hutagalung (1988) which shows that the negative impact of PKC on protozoa had been indicated in cattle fed a PKC-based diet. Some dietary factors may decrease or even remove ruminal protozoa. In addition, Abdullah et al. (1995) stated that the number of protozoa in the rumen fluid of sheep was reduced after consuming PKC in the first two groups of sheep. Regarding, the reduction in the number of protozoa was related to the lowest butyrate production. In addition, a study had reported that unsaturated fatty acids reduce protozoa numbers (Machmüller and Kreuzer 1999) due to the fact that unsaturated C18 fatty acids are toxic to protozoa. Therefore, the use of PKC based diets may have the potential of reducing protozoa numbers thereby changing the ruminal ecosystem, and indirectly increase the bacterial population and activity. However, the protozoa and, hence the production of sulphide, decreases the bioavailability of dietary Cu but our findings show that protozoa levels declined at the higher levels of corn as energy source which is deemed a motivator for protozoa growth (Saeed et al. 2018).

Fatty acid composition of rumen liquor

The main fatty acids substrate for biohydrogenation in ruminants is linolenic acid (cis-9,cis-12,cis-15-18:3) because it is the most abundant fatty acid present in glycolipids and phospholipids of feed. The current study found that the proportion of fatty acids were not affected by corn dietary intake at 24 and 72 h, this could be due to the composition of fatty acids in these diets. The lower of C18:1n9 in T2 and T3 at 24 h was similar with findings of Adeyemi et al. (2015) was noted that stearic acid (C18:0) is the primary end product of rumen biohydrogenation. Significant reduction of C18:0 could be due to incomplete biohydrogenation of C18:2n6c, C18:1n9c and C18:3n-3 which yielded a higher concentration of biohydrogenation intermediates *trans*-11 C18:1 and CLAc9t11. C18:1n9 declined significantly with increasing levels of corn for this study at 24 and 72 h. This is contrary to what we have expected

and may be due to the ability of rumen bacteria to synthesize the biohydrogenation intermediate from C18:2n-6 (Harfoot and Hazlewood 1997). In this study palmitic (C16:0) was the second most abundant ruminal FA at 72 h although its proportion was heavily influenced by corn diets. A possible explanation for this may be that palm oils and its byproducts are usually considered good source of C16:0 (Abubakr et al. 2015). The control diet had a lower proportion of C18:1 *trans*-11 at 72 h than other treatments. It was established that these unsaturated fatty acids includes CLA and vaccenic acid (Devillard et al. 2006), further increasing the possible significance of protozoa in the delivery of health-promoting FA from the rumen. Beam et al. (2000) reported that the extent of biohydrogenation increases in tandem with the degree of unsaturation of fatty acids. The increasing levels of substituted corn could probably be responsible for the significantly lower concentrations of C18:1n9c. It has been established that the incomplete biohydrogenation of linoleic acid (Harfoot and Hazlewood 1997; Jenkins et al. 2008), linolenic acid , and oleic acid yielded *trans* 11-18:1, CLAc9t11, CLAc12t10 and other conjugated isomers. Protozoa play a significant role in biohydrogenation of the rumen microbial FA and contain proportionally more unsaturated fatty acid, and CLA (Jayanegara 2014). Thus, protozoa could represent a primary source of CLA and help increase the concentration of CLAc9 *trans*-11 as results showed at 24 h. It is well-known that protozoal lipids contain proportionally more unsaturated FA than the bacterial fraction (Jayanegara 2014; Saeed et al. 2018).

In this study**,** the substitution of different levels of corn in the PCK basal diet increased CH_4 emissions and the biohydrogenation percentage of LA and C18 PUFA under *in vitro* conditions. The corn substitution improved fermentation characteristics without any adverse effect on total gas production *in vitro*. It also affected the relative abundance of total bacteria. Short-chain fatty acid absorption seems to help in stabilizing ruminal pH by eliminating the effect of toxic in the rumen.

Declarations

Ethics approval

None

Author contributions

All authors were contributed to the analyzed the data and writing of the present article.

Conflicts of interest

The authors declare no conflicts of interest.

Consent for publication

Not applicable

Availability of data and materials

Availability of data and materials used and analyzed during this study is available from the corresponding author on reasonable request.

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Tables

Table 1 Ingredients and chemical compositions of the treatment diets (DM basis)

Item	corn concentration (%)		
	T1	T2	T3
Rice straw urea treated	20	20	20
PKC	75.3	70.3	65.3
Protected fat (Megalac)	3	3	3
Corn	0	5	10
$CaCO_3$	1	1	1
NaCl	0.5	0.5	0.5
Vitamin premix	0.2	0.2	0.2
Total	100	100	100
Chemical composition:			
DM	91.78	91.66	91.55
Ash	13.80	12.72	12.74
OM	86.19	87.27	87.26
CP	15.42	14.88	14.09
EE	5.3	5.1	4.33
CF	26.6	24.50	20.83
NDF	62.36	60.06	55.66
ADF	45.60	40.96	37.30
ADL	6.56	6.10	5.43
Gross energy (MJ/kg DM)	16.89	17.29	17.65
Calculation:			
Hemicellulose	16.76	19.10	18.36
Cellulose	39.03	34.86	31.86
NFE	40.44	41.11	48.39
ME (MJ/Kg DM)	7.36	8.23	8.92
TDN (%)	43.91	49.24	53.46

The Ratio of Concentrate to Straw were given on 80 : 20 dry matter basis, vitamin premix A: 10,000,000 IU; Vitamin E: 70,000 IU; Vitamin D: 1,600,000 IU; DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; CF: crude fiber; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; NFE: nitrogen-free extract; ME: metabolizable energy; TDN: total digestible nutrients

Table 2 Kinetics gas production volume at different incubation times for experimental treatments

Parameters		T1	T2	T3	SEM	*P*-value
Kinetics gas production						
a	(mL)	36.00	35.20	36.33	0.37	0.49
b	(mL)	40.51	45.08	42.37	0.84	0.06
c	(mL/h)	0.045	0.050	0.045	0.001	0.25
a+b	(mL)	76.51	80.28	78.71	0.84	0.16

T1:(75.3% PKC + 0% corn), T2:(70.3% PKC + 5% corn), T3:(65.3% PKC + 10% corn)

Table 3 Effect of variations among treatment diets on some fermentation characteristics

Parameters	T1	T2	T3	SEM	*P*-value
IVDMD	51.78	44.37	49.91	2.06	0.31
IVOMD	80.54	85.04	82.97	1.82	0.61
CH_4 (mmol/L of gas)	2.81^{b}	2.71^{b}	3.72^{a}	0.16	0.01
NH_3-N (mg/dL)					
0 h	23.76	23.86	25.46	1.98	0.67
72 h	36.79	35.69	32.79	1.78	0.95
pH					
0 h	6.80	6.69	6.75	0.02	0.34
72 h	6.83	6.79	6.81	0.01	0.67

T1:(75.3% PKC + 0% corn), T2:(70.3% PKC + 5% corn), T3:(65.3% PKC + 10% corn), ^{a,b} Means in the same row with different superscripts are significantly different.

Table 4 Production of volatile fatty acids (VFA, mmol/ml) after 72 h *in vitro* fermentation of different levels of corn substitution

Parameter	T1	T2	T3	SEM	*P*-value
Total VFA					
0 h	12.77	12.87	15.45	0.45	0.42
72 h	13.61	14.67	16.86	0.51	0.51
Mean	13.40	13.95	16.30	0.79	0.29
Acetate					
0 h	6.11	6.19	7.53	0.10	0.44
72 h	7.20	6.48	7.99	0.04	0.51
Mean	6.88	6.40	7.86	0.39	0.31
Propionate					
0 h	3.56	4.05	5.23	0.64	0.65
72 h	2.94^{b}	3.12^{ab}	4.53^{a}	0.31	0.05
Mean	3.05^{b}	3.47^{ab}	4.84^{a}	0.31	0.5
Isopropionate					
0 h	0.91	1.04	0.93	0.32	0.89
72 h	1.09^{a}	1.06^{a}	0.84^{b}	0.18	0.05
Mean	1.04	1.05	0.87	0.04	0.21
Butyrate					
0 h	2.95	3.62	3.47	0.48	0.93
72 h	2.22^{b}	2.62^{ab}	3.52^{a}	0.22	0.05
Mean	2.33	3.02	3.50	0.23	0.16
Isobutyrate					
0 h	2.49	2.07	2.05	0.91	0.89
72 h	2.01	2.08	2.64	1.13	0.34
Mean	2.13	2.07	2.40	0.16	0.68
C2: C3					
0 h	1.64	1.83	1.83	0.07	0.64
72 h	2.06	1.75	2.06	0.07	0.20
Mean	1.95	1.78	1.79	0.05	0.44

T1:(75.3% PKC + 0% corn), T2:(70.3% PKC + 5% corn), T3:(65.3% PKC + 10% corn), [a,b] Means in the same row with different superscripts are significantly different.

Table 5 Effect of dietary treatments of sampling on microbial population (copies/ ml) *in vitro* trial

Item	Diets				
Species	T1	T2	T3	SEM	*P*-value
Total bacteria ($\times 10^{10}$)					
0 h	11.03^{b}	11.64^{a}	11.67^{a}	0.09	0.001
24 h	11.58	11.60	11.29	0.06	0.08
Mean	11.30^{b}	11.62^{a}	11.48^{ab}	0.05	0.05
F. succinogenes($\times 10^{9}$)					
0 h	7.15	8.17	7.94	0.20	0.09
24 h	7.75	8.01	7.50	0.16	0.46
Mean	7.45	8.09	7.72	0.12	0.12
R. albus ($\times 10^{6}$)					
0 h	7.70	6.84	6.71	0.22	0.13
24 h	7.86	6.77	7.78	0.30	0.27
Mean	7.78	6.81	7.24	0.18	0.10
R. flavefaciens($\times 10^{7}$)					
0 h	6.87	7.08	7.16	0.12	0.66
24 h	6.84	7.46	6.91	0.14	0.17
Mean	6.85	7.27	7.03	0.09	0.21
Methanogenic archaea ($\times 10^{9}$)					
0 h	4.97^{a}	3.92^{b}	3.74^{b}	0.17	0.001
24 h	5.56^{a}	4.12^{b}	5.12^{a}	0.18	0.001
Mean	5.27^{a}	4.02^{b}	4.43^{b}	0.14	0.001
Total protozoa ($\times 10^{5}$)					
0 h	3.40^{b}	5.17^{a}	4.36^{ab}	0.27	0.01
24 h	4.37^{a}	5.72^{a}	3.87^{b}	0.24	0.001
Mean	3.88^{b}	5.39^{a}	4.12^{ab}	0.18	0.001

T1: (75.3% PKC + 0% corn), T2: (70.3% PKC + 5% corn), T3: (65.3% PKC + 10% corn), [a,b] Means in the same row with different superscripts are significantly different.

Table 6 Fatty acid composition (g/100 g total fatty acids) of rumen fluid and rate of biohydrogenation at 24 h incubation

Fatty acids	T1	T2	T3	SEM	*P*-value
C12:0	4.32	4.91	3.66	0.33	0.39
C14:0	2.08	2.19	2.15	0.03	0.55
C15:0	0.70	0.73	0.54	0.09	0.73
C15:1	0.89	0.96	0.81	0.09	0.85
C16:0	16.37	19.19	19.88	0.71	0.06
C16:1n-7	0.65	0.55	0.66	0.05	0.67
C16:1n-9	0.45[a]	0.12[b]	0.48[a]	0.07	0.001
C18:0	26.25	26.66	28.69	0.95	0.59
C18:1n9	22.96	23.16	27.80	1.11	0.12
C18:1 *Trans*-11	4.46	3.52	3.75	0.36	0.60
C18:2n-6	15.60	13.16	14.04	0.85	0.56
CLAc9 *Trans*-11	0.64[b]	0.68[b]	1.20[a]	0.11	0.05
CLAc12 *Trans*-10	0.95	1.40	0.50	0.18	0.10
C18:3n-3	1.34	1.44	1.49	0.04	0.43
C20:4n-6	0.50	0.51	0.55	0.01	0.52
C20:5n-3	1.84[a]	1.46[a]	0.45[b]	0.22	0.001
C22:5n-3	0.33	0.33	0.31	0.008	0.67
C22:6n-3	0.88	0.79	0.77	0.06	0.81
ΣSFA	49.73	53.69	48.61	1.43	0.35
ΣUFA	50.27	46.30	51.39	1.43	0.35
ΣMUFA	29.32	28.32	33.40	1.13	0.15
ΣPUFA n-3	4.41[a]	3.58[ab]	2.60[b]	0.29	0.01
ΣPUFA n-6	16.10	13.67	14.60	0.85	0.56
ΣPUFA	20.51	17.25	17.20	0.99	0.33
Σ *Trans* FA	4.46	3.52	3.75	0.36	0.60
ΣCLA	1.32	1.69	1.60	0.19	0.77
n-6 : n-3	3.64	3.88	5.71	0.46	0.12
UFA:SFA	1.01	0.86	1.08	0.06	0.38
Poly: SFA Ratio	0.41	0.32	0.36	0.02	0.47
Apparent biohydrogenation (%)					
C18:1n9	15.51[a]	9.38[ab]	1.31[b]	2.42	0.05

C18:2n-6c	20.58	33.00	37.70	3.70	0.14
C18:3n-3	60.51	57.47	56.06	1.31	0.42

MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; UFA, unsaturated fatty acids. [a,b] Means in the same row with different superscripts are significantly different.

Table 7 Fatty acid composition (g/100 g total fatty acids) of rumen fluid and rate of biohydrogenation at 72 h incubation

Fatty acids	T1	T2	T3	SEM	*P*-value
C12:0	3.53[a]	2.24[b]	3.05[a]	0.24	0.05
C14:0	2.62	2.74	2.97	0.07	0.12
C15:0	2.51	2.81	2.94	0.11	0.34
C15:1	0.91[b]	1.24[a]	1.19[a]	0.06	0.05
C16:0	16.96[b]	24.34[a]	23.56[a]	1.49	0.001
C16:1n-7	1.17	1.44	0.79	0.18	0.46
C16:1n-9	1.16	0.60	1.14	0.16	0.34
C18:0	49.64[a]	39.68[b]	40.20[b]	2.19	0.05
C18:1n9	3.32[b]	4.91[a]	4.73[a]	0.32	0.001
C18:1 *Trans*-11	1.89[b]	3.05[a]	2.77[a]	0.23	0.05
C18:2n-6	10.27	12.56	10.91	0.66	0.43
CLAc9 *Trans*-11	1.39	1.12	1.39	0.18	0.85
CLAc12 *Trans*-10	0.81	1.11	0.91	0.10	0.59
C18:3n-3	1.23	0.93	1.53	0.23	0.69
C20:4n-6	0.60[b]	0.90[a]	0.59[b]	0.07	0.07
C20:5n-3	1.40	0.55	1.21	0.21	0.27
C22:5n-3	0.55[a]	0.30[b]	0.28[b]	0.05	0.05
C22:6n-3	0.91	0.69	0.99	0.08	0.44
ΣSFA	75.26	71.83	72.72	0.93	0.37
ΣUFA	24.73	28.16	27.27	0.93	0.37
ΣMUFA	8.46[b]	11.24[a]	10.64[a]	0.54	0.01
ΣPUFA n-3	4.10	2.48	4.02	0.37	0.10
ΣPUFA n-6	10.87	13.46	11.50	0.72	0.39
ΣPUFA	14.97	15.94	15.52	0.60	0.87
Σ *Trans* FA	1.89[b]	3.05[a]	2.77[a]	0.23	0.05
ΣCLA	2.20	2.23	2.31	0.20	0.98
n-6 : n-3	2.63[b]	5.43[a]	2.94[b]	0.59	0.05
UFA:SFA	0.33	0.39	0.37	0.01	0.35
Poly: SFA Ratio	0.20	0.22	0.21	0.009	0.69
Apparent biohydrogenation (%)					
C18:1n9	87.01[a]	80.79[b]	81.50[b]	1.25	0.001

C18:2n-6c	47.73	36.05	44.44	3.36	0.43
C18:3n-3	63.65	72.63	54.82	4.17	0.23

MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; UFA, unsaturated fatty acids. [a,b] Means in the same row with different superscripts are significantly different.

Figures

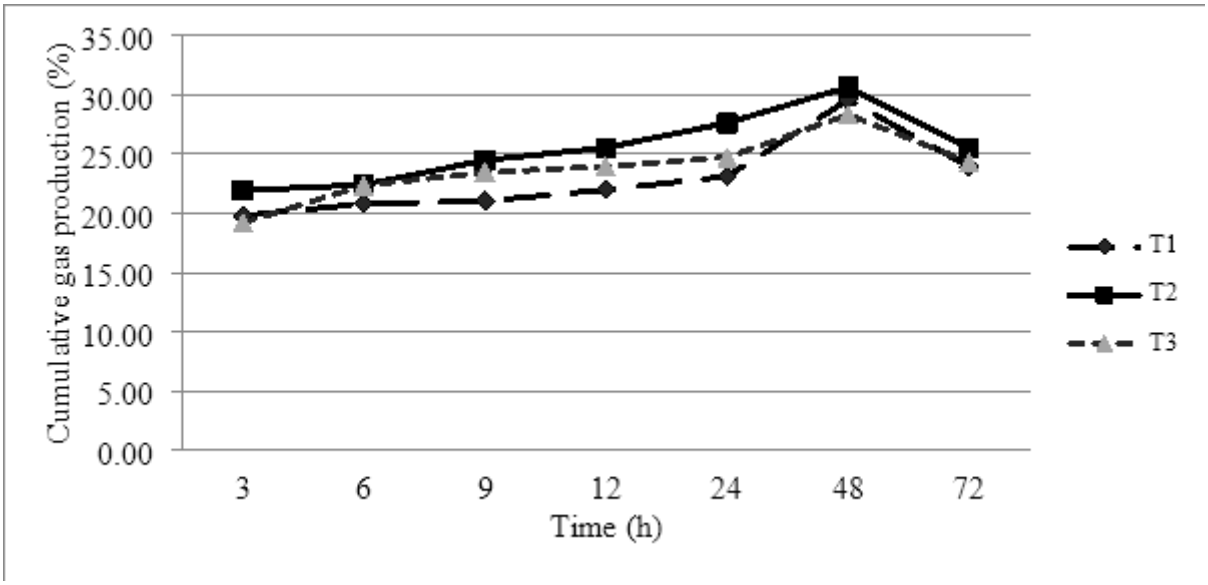

Figure 1

Cumulative gas production in different incubation time at various level of corn, (♦ =T1: 75.3% PKC + 0% corn), (■ = T2: 70.3% PKC + 5% corn), (▲= T3: 65.3% PKC + 10% corn).