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In Vitro Fermentation Characteristics of Camelina Meal Comparison with Soybean Meal

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Abstract

The search for new and cheap sources of protein has been increased lately. Although camelina meal has antinutritive factors; compare to soybean it can be widely useable. The objective of this study is to remove the question mark in minds about camelina meal and to determine the fermentation characteristics parameters including pH, ammonia-N level, volatile fatty acids concentration as well as total gas volume, methane proportion and the estimated degradation of camelina meal in comparison with soybean meal. Basically, we used *in vitro* gas production system according to modified Hohenheim Gas Test (HFT) to compare camelina meal and soybean meal. Rumen contents obtained from two Holstein cows. There was no significant difference of pH and ammonia-N concentration between soybean meal and camelina meal, whereas total volatile fatty acid and acetate concentration were reduced in camelina meal. Additionally, total gas production, fermentative CO_2 and estimated ME and organic matter digestibility were not altered. However, methane production decreased significantly in camelina meal fermenters. Consequently, it was concluded that camelina meal can be replaced of soybean meal, since microbial fermentation does not change and it might reduce the methane emission in which has commonly major effect on environmental pollution as a sera gas.

Introduction

Soybean meal (SBM) is commonly used in livestock nutrition as an attractive protein source of plant origin in the world, although its high price. In ruminants that costs don't compete with the humans or monogastric animals have encouraged the search alternative protein sources to replace soybean meal (Haddad, 2006; Alves *et al.*, 2016; Florou-Paneri *et al.*, 2014). In the last decades, due to its high quality protein and the search for cheaper resources, the demand for camelina seeds has increased (Russo *et al.*, 2017). *Camelina sativa* compared to soybean has low nutrient requirements, good resistance to diseases and pests (Halmemies-Beauchet-Filleau *et al.*, 2018). Camelina meal (CM), the by-product of camelina oil extraction, is an alternative protein source for livestock despite its higher antinutritive factors compared to soybean meal (Sizmaz *et al.*, 2016; Russo *et al.*, 2017). Nevertheless, CM has been considered as acceptable (Waraich *et al.,* 2013). CM in livestock diets contain glucosinolates, phytic acid, sinapine and condensed tannins. Especially glucosinolates are antinutritional factors; disrupts the thyroid activity and decreases the feed intake (Paula *et al.,* 2019). Therefore, in 2002, European Union (EU) Directive forbid the usage of *C. sativa* in livestock rations due to the presence of glucosinolates. Yet, in 2008 EU Directive, after many studies, permits the feed use of *C. sativa* and its derivatives (Colombini *et al.,* 2014). Because ruminants are more tolerant to glucosinolates compared to monogastric animals; is also a reason to put them back in the field (Vincent *et al.,* 1988).

We hypothesized that the camelina meal might be shown similar fermentation characteristics with soybean meal. Thus, the current study is conducted to investigate the *in vitro* rumen fermentation parameters including pH, ammonia-N level, volatile fatty acid concentration, estimated degradation and gas

production of camelina meal as a replacement of soybean meal.

Materials and Methods

Based on our previous study (Sizmaz *et al.,* 2016), evaluating the impact of nutrients degradation of camelina and soybean meal *in vitro*, the fermentation characteristics, gas production and fermentative methane emission were investigated in this study. The same camelina meal and soybean meal samples were used in this experiment. Thereby the nutrients of the samples were taken from our previous study (Table 1).

In Vitro Fermentation Technique

In vitro rumen fermentation was performed according to a modified HFT (Menke and Steingass, 1986). Two hundred milligrams of the camelina meal and soybean meal substrate were incubated with 30 ml of a ruminal buffered suspension (2:1; buffer solution: rumen fluid) by flushing CO_2 before was anaerobically dispensed in each syringe at 39°C. The rumen contents were obtained from two cannulated Holstein cows with a live weight of 630 ± 21.3 kg before morning feeding kept at Ministry of Agriculture and Forestry, International Center for Livestock Research and Training. Rumen fluid was immediately transferred to the laboratory to *in vitro* fermentation with preheated thermos flask. Then, rumen fluid immediately mixed with the buffer solution (Macro Element Solution: Na_2HPO_4, KH_2PO_4 and $MgSO_4.7H_2O$; Micro Element Solution: $CaCl_2.2H_2O$, $MnCl_2.4H_2O$, $CoCl_2.6H_2O$ and $FeCl_3.6H_2O$; Buffer Solution=$NaHCO_3$ and NH_4HCO_3; Resazurin Solution=Resazurin; Reductant Solution= $Na_2S.7H_2O$ and NaOH) which was bubbled with CO_2, at 39°C for 24h incubation.

Rumen Sampling and Analysis

After 24h incubation, the rumen fluid samples were collected from syringes of each group and were strained into the individual beakers with a sterile cheesecloth to stop the fermentation. The pH was measured immediately with a pH-meter (Hanna Instruments). Ammonia-N in rumen fluid was analyzed using spectrophotometry by using indophenol blue method at 546 nm according to the method described by Chaney and Marbach (1962).

Concentration of VFA were determined according to Geissler *et al.* (1976). Rumen samples were centrifuged at 4.000 rpm for 15 min at 4°C. One ml of supernatant was then transferred to an Eppendorf tube and mixed with 0.2 ml ice-cold 25% met phosphoric acid solution. Then, tubes were kept at 4°C for 30 min. Subsequently, these tubes were centrifuged again at 13.000 rpm for 10 min at 4°C and the supernatant was transferred into gas chromatography vials to determine acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid and isovaleric acid concentrations. Samples were analyzed by using gas chromatography (Shimadzu GC-2010, Shimadzu Co., Kyoto, Japan) coupled with a capillary column (TR-151035, TRB-FFAP, 30 m x 0.53 mm). The column temperature was programmed to increase gradually from 120°C to 160°C during the analysis. In addition, the injector port and flame ionization detector (FID) temperatures were fixed at 230°C and 250°C, respectively. The injection volume was set to 1 µL and analyses were performed in duplicate.

In Vitro Total Gas Volume, Methane Production and Estimated Digestion Values

After 24h of incubation, the total gas volume of each syringe was recorded. The metabolizable energy (ME) and organic matter digestibility (DOM) contents of the camelina meal and soybean meal were calculated using the equations by Menke and Steingass (1988) as follows:

$$ME\ (MJ/kg) = 2.20 + 0.136 \times Gas24h + 0.057 \times CP$$

$$DOM\ (g/kg) = 14.88 + 0.889 \times Gas24h + 0.45 \times CP + 0.0651 \times A$$

Where; Gas24 h net gas production (ml/200mg), CP; crude protein (%), A; ash content (%).

Methane production was calculated using the equations proposed by Abdl-Rahman (2010) based on the stoichiometry of Wolin (1960), as follows;

$$Fermentative\ CO_2 = A/2 + P/4 + 1.5\ B$$

$$Fermentative\ CH_4 = (A + 2\ B) - CO_2$$

A; mole of acetate, P; mole of propionate, B; mole of butyrate.

Table 1. The chemical composition of camelina meal and soybean meal used in the experiment.

Chemical Composition	Soybean Meal	Camelina Meal
DM	896.00	885.90
OM	940.00	946.10
CP	482.00	369.70
EE	16.50	14.90
CF	52.50	110.70
Ash	60.00	53.90
ME, MJ/kg	11.67	10.40

DM; Dry matter, OM; organic matter, CP; crude protein, EE; ether extract, CF; crude fiber, ME; metabolizable energy.

Statistical Analysis

Statistical analysis for the data from the rumen fermentation parameters were conducted using SPSS software (V22.0; SPSS Inc., Chicago, IL, USA). First, the Shapiro–Wilk test was adopted to check whether the distribution of the variables exhibited a normal distribution. Then, the variables that showed a normal distribution were analyzed by the independent sample t test. Significant differences were declared at $P < 0.05$; a tendency was considered for $0.05 < P \leq 0.10$.

Results

The *in vitro* fermentation characteristics of the soybean meal and camelina meal are shown in Table 2. Basically, after the results from *in vitro* fermentation; the levels of acetate and total VFA are significantly higher in soybean meal than CM ($P < 0.05$). Additionally, as it can be seen in the Table 2 the level of isovalerate and propionate tended to increase in soybean meal than CM ($0.05 < P \leq 0.10$). Soybean meal's pH level were higher than CM numerically (6.92 vs. 6.79). However, as for A/P, isobutyrate, butyrate, isovalorate and valorate levels; no significant difference was observed between soybean meal and CM ($P > 0.05$).

The *in vitro* total gas volume, fermentative CH_4, fermentative CO_2 and estimated digestibility of camelina meal and soybean meal are shown in Table 3. Initially the level of fermentative CH_4 is significantly higher in soybean meal than CM ($P < 0.05$). However, there were no differences determined in the fermentative CO_2, total gas volume, ME and DOM between soybean meal and CM ($P > 0.05$).

Discussion

In the recent years, camelina meal has been evaluated for alternative protein sources in ruminant rations. Plenty of studies have shown the potential of camelina meal to improve the degradability and few of them to modify ruminal fermentation (Moriel *et al.,* 2011; Colombini *et al.,* 2014; Lawrence *et al.,* 2016; Sizmaz *et al.,* 2016 & Brando *et al.,* 2018). Present study contributes the literatures for an *in vitro* fermentation characteristics including pH, ammonia level, VFA concentration, total gas volume and methane production of CM compared to SBM in an *in vitro* gas production system. Our study provided that pH, ammonia level and VFA were not altered while CM had less concentration of acetate and total VFA. This could be caused by the fiber content of SBM and CM and would be effective *in vivo* studies. Feeding CM at 10% of the diet to heifers did not affect ruminal pH and ammonia level and volatile fatty acids concentration (Lawrence *et al.,* 2016). Brando *et al.* (2018) reported that the ruminal pH and total VFA concentration were not affected by CM treatment at level of 50% and 100% in fermentor system. These lack of effects of CM on ruminal fermentation characteristics may be related to the lack of effects on ruminal microbial population; bacteria, fungi, and protozoa (Bayat *et al.,* 2015; Halmemies-Beauchet-Filleau *et al.,* 2016 & Paula *et al.,* 2019). Paula *et al.* (2019) stated that none of the reported studies that tested CM observed effects on total VFA concentration. Additionally, the study by Lawrence *et al.* (2016) found that the NH_3-N levels was higher in CM compared with DDGS and linseed meal fed heifers and Brandao *et al.* (2018) has shown that the ammonia level decreased by inoculation of CM to the fermenters. These results associated with the bacterial population and activity, would indicate that the protein degradation in the ration.

Because of the *in vitro* and *in vivo* conditions, protein sources, dosage of the CM, the forage:concentrate ratio and basal diet composition, the CM does not alter on overall microbial fermentation but may effect ruminal milieu as a bacterial community composition and thus change ammonia level and the VFA molar proportions.

Table 2. The *in vitro* pH, ammonia-N (mmol/l) and volatile fatty acids concentration (mM/l) of camelina meal (CM) and soybean meal (SBM).

Treatments	pH	Ammonia-N	A/P	Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate	Valerate	Total VFA
SBM	6.92± 0.194	41.90± 3.119	3.05± 0.152	42.91± 1.276	14.10± 0.285	1.95± 0.111	8.17± 0.696	3.28± 0.265	2.18± 0.063	72.58± 0.67
CM	6.79± 0.147	39.04± 1.986	2.88± 0.067	35.79± 0.883	12.47± 0.595	1.75± 0.047	7.51± 1.125	2.59± 0.126	1.99± 0.059	62.11± 2.489
P	0.622	0.483	0.358	0.010	0.068	0.181	0.649	0.078	0.101	0.015

Table 3. The *in vitro* total gas volume, CO_2 and CH_4 proportion (mM/L) and estimated ME (MJ/kg) and degradation of organic matter (DOM; g/kg) of camelina meal (CM) and soybean meal (SBM).

Treatments	Fermentative CO_2	Fermentative CH_4	Total gas volume	ME	DOM
SBM	37.23±0.479	22.01±0.361	56.67±17.487	6.49±0.474	47.04±3.111
CM	32.28±2.278	18.54±0.857	71.33±32.338	6.25±0.881	44.55±5.752
P	0.101	0.020	0.710	0.820	0.723

In the present study, we evaluated the decreasing effect of CM on *in vitro* methane production. Our best knowledge is this is the first trial of CM effect on methane emission. Some studies have been conducted the effects of camelina oil on total gas volume, CO_2 and methane production (Bayat *et al.,* 2015; Ebeid *et al.,* 2020) that reported similar findings with our experiment. In these studies, the authors reported that no difference has been found in the gas volume and CO_2 concentration among treatments and a decrease in methane emission in lactating dairy cows fed with different forage:concentrate ratio and camelina oil concentrations and different basal diet compositions such as supplemented with feed additives. Camelina seeds showed the decreasing effect on methane in a ration having a roughage-to-concentrate ratio (Wang *et al.,* 2017). Therefore, the important point the effects on methane emission is the dietary form of camelina if seed, oil or meal used in the diet.

Zagorakis *et al.* (2015) and Sizmaz *et al.* (2016) reported that CM has the potential to substitute SBM, with the protein having relatively low effective degradability compared with that of SBM. Therefore, Hao *et al.* (2020) reported that the effectiveness of total diet degradation rate of CP was decreased linearly while DOM and gross energy degradation were increased in flax seed meal. On the contrary, in the trial conducted by Salas *et al.* (2019) the *in vitro* OM degradation was decreased compared with SBM. According to the study of Brando *et al.* (2018), the degradation of OM was not affected by supplemented camelina. In the present study the estimated degradation of ME and OM were not altered in the treatment groups. The digested energy of CM likewise SBM is an important reason to improve the performance in ruminants. As has been argued previously in the specific case of the comparison between CM and SBM, the form of the camelina, chemical oil extraction process and the type of degradation of CM *in vitro* or *in situ* could modify the rumen fermentation and alter the degradation.

There is lack of evidence concerning the effect of CM on ruminal OM and ME degradation as well as methane emission can be explained by the fact that just maintained *in vitro* method in the present study. One reason might be the relationship of oil extraction way in feedstuffs and the conditions during the *in vitro* trial such as bag characteristics, incubation condition in the rumen. The possible effect that there is lack of *in vitro* investigation that will support the microbial fermentation characteristics of CM cannot be excluded.

Conclusion

The results of the present study showed that replacing a proportion of SBM with CM in an *in vitro* rumen fermentation can increase the proportion of acetate and total VFA and decrease the methane production whereas the total gas volume was not affected. The other fermentation characteristics and the estimated degradation of ME and OM were not altered. Thus, we considered that CM can be replaced by SBM, when used as a main protein source in isocaloric and isonitrogenous diets. Thereby, CM could be an alternative protein source for ruminant diets.

Conflict of Interest

The authors declare no conflict of interest.

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