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Impact of Supplementation of Hemp Seeds (*Cannavis sativa* L.) on *In Vitro* Gas Fermentation Parameters and Digestibility using Foregut Fluid from Dromdary Camel

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Abstract This experiment was conducted to investigate the effect of hemp seeds on *in vitro* biogas production, and digestibility of one-humped camel. Two female fistulated dromedary camels (300 kg) were fed with a forage diet (60 wheat straw and 40 alfalfa) for 1 month. Then rumen fluid was supplied from camels prior to the morning meal. Experimental samples were control diet and diet containing 10 % hemp seeds. Glass vials 100 ml containing 30 ml buffered rumen fluid was used. Gas production parameters were measured by exponential models and digestibility of foregut bacteria of camels were determined. The results showed that addition hemp seeds decreased potential of gas production ($P<0.05$), but gas production rate was not influenced ($P>0.05$). Actually digested organic matter and microbial biomass of diet containing hemp seeds were more than a control diet ($P<0.05$).While, hemp seeds supplementation had the greatest digestibility by cellulolytic bacteria of camel, after 24, 48 and 72 h incubation. The results also showed hemp seeds decreased ammonia nitrogen concentration as compared to the control treatment ($P<0.05$). Therefore, adding hemp seeds in the camel diet caused to reduce gas emission and ammonia nitrogen production in foregut and increased cellulolytic bacteria digestibility; so hemp seeds may be used in camel diets and will influence camel growth performance and decrease gas emissions in the environment.

Keywords: Hemp seeds, Gas emission, *In vitro* digestibility, Dromedary camel.

Introduction

Methane and carbon dioxide are natural ending-product of rumen fermentation and has global warming potential (Busquet *et al.*, 2005). In this case also one of the promising strategies is to manipulate the biochemical pathways in the rumen to produce less methane by herbs; and phenolic compounds and tannin contents of these herbs, decrease methane production in the rumen (Busquet *et al.*, 2005). Therefore, some medicinal plants as rumen fermentation variables may improve the energy efficiency in the rumen and ultimately affect animal productivity (Busquet *et al.*, 2005). The using of *Malva sylvestris* caused to increase of dry matter and neutral detergent fiber digestibility in dromedary camels (Mohammadabadi & Chaji, 2019). The lignified pasture forage may negatively affect the plant quality and nutrients digestibility (Zandi Esfahan *et al.,* 2010). Scientists are interested in exploring the use of medicinal plants to manipulate rumen fermentation, improve rumen ecology, and improve the use of nutrients in domestic animals (Busquet *et al.,* 2005).

During recent years, researchers and producers have focused on the use of feed additives and phytochemicals that can have therapeutic properties and have a positive impact on animal and human health. Hemp seeds (*Cannabis sativa)* is belong to the Cannabaceae family and containing some nutrients like proteins, amino acids and essential fatty acids (Mahmoudi *et al.,* 2012). The dry matter, crude protein, ether extract, ash and metabolizable energy of hemp seeds were 95, 22.5, 33, 4.56 % and 4.5 Mcal/kg DM (Karmshahi *et al.,* 2019). The yield of fiber varieties is more than 20 tons of dry matter per hectare (Mahmoudi *et al.,* 2012). This variety produces taller and less seeds than oil variety (Callaway, 2004).

The crude protein content of oil varieties is 26.5% and crude protein content of fiber varieties is 26.6% (Silversides & Lefrancois, 2005).Studies have shown that hemp seeds protein is rich in methionine and cysteine (Odani & Odani, 1998). The researchers found that the amount of seeds oil varied between 26.3% and 37.5%, and typically about 90% of seeds fatty acids were polyunsaturated fatty acids like linoleic acid (alpha-linolenic acid) (Callaway, 2004). The n6/n3 ratio in hemp seed oil is normally between 2:1 and 3:1, which is considered to be proper for human and animal health (Callaway, 2004). Hemp seeds have a favorable amino acid pattern, are resistant to rumen degradation and therefore have high accessibility throughout the gastrointestinal tract (Mustafa *et al.,* 1999).

In the experiment by Gibb *et al.* (2005), it is concluded that feeding hemp seeds had no effect on dry matter intake and daily weight gain of feedlot steers. However, conjugated linoleic acid and omega-3 fatty acids of carcass adipose tissue increased without adverse effects on performance (Gibb *et al.,* 2005). In experiments on cattle and sheep, hemp seeds have been shown to be a good source of non-digestible crude protein (Callaway, 2004). Hemp meal can be used in sheep up to 20% of the diet without harmful effects on other nutrient use (Mustafa *et al.,* 1999).

Plant secondary metabolites such as tannins can modify rumen fermentation to enhance the efficiency of the utilization of feed energy and decrease methane emissions (Broudisco & Pochnet, 1994). Therefore, this experiment was aimed to investigate the effects of the hemp seeds on the in vitro rumen fermentation, gas emission and digestibility using foregut rumen fluid from dromedary camel.

Materials & Methods

Two fistulated females dromedary camels (300 kg, 2 years old, Arabian) were fed with wheat straw, alfalfa hay and some hemp seeds for 1 month (crude protein 7.5 %, dry matter 85 %, organic matter 81%, crude fiber 62%, nautral detergent fiber (NDF) 68 % and acid detergent fiber (ADF) 45%). Then foregut fluid was supplied from camels prior to the morning meal, homogenized in a laboratory blender, filtered through three layers of cheese-cloth and purged with CO_2. Experimental samples were control diet or without hemp seeds (60 % wheat straw and 40 % alfalfa) and diet containing hemp seeds (55 % wheat straw and 35 % alfalfa and 10 % hemp seed). About 35 ml buffered rumen fluid added to 200 ± 10 mg milled sample (1.0 mm screen) and were incubated under CO_2 reflux in glass vials for 2, 4, 6, 8, 10, 12, 16, 24, 48, 72 and 96 h, in 39°C (Menk & Steingass, 1988). Cumulative gas production data were fitted to the exponential equation $Y=b\ (1-e^{-ct})$, where b is the gas production potential (ml), c is the gas production rate (ml/h), t is the incubation time (h) and Y is the gas produced at time t (Ørskov & McDonald, 1979). At the end of incubation, the vial's content was transferred into an Erlenmeyer flask, boiled with neutral detergent solution, then filtered, dried (60 °C for 48 h) and ashed (550 °C for 3 h). The partitioning factor (PF), microbial biomass and actually digested organic matter were estimated according to Blummel *et al*. (1997). The activity of dromedary camel’s foregut bacteria in the specific culture medium was determined according to Mohammadabadi & Chaji (2011). Culture glasses containing 1 g sample were autoclaved at 120 °C for 15 minutes. Foregut fluid centrifuged at 1000 rpm for 10 min and the supernatant was used in bacteria medium under anaerobic condition. The medium was included cellobiose, sodium sulfide, sodium carbonate, fungicides (benomyl and metalaxyl), cysteine-HCl, peptone, trypticase, and yeast extract mixture. The amount of 36 ml culture medium and 4 ml camel’s foregut fluid was inoculated into culture glasses and were incubated at 39 °C for 24, 48 and 72 h and the digestibility of dry matter and organic matter for each incubation time were measured. The content of each vial was centrifuged at 1500 rpm for 20 min and the residuals were collected and dried. Digestibility of dry matter and organic matter was calculated by difference between weights of primary substrate from weight after incubation. After incubation times, the glasses content was used for the determination of pH by using pH meter (Meter Model 691, Switzerland). Ammonia nitrogen concentration of glasses was measured using the phenol-hypochlorite method (Broderick & Kang, 1980). Data were analyzed as a completely randomized design (t-test) using the General Linear Model (GLM) procedure of the SAS (SAS, 2003) based on the statistical model: $Y_{ij}= \mu + T_i + e_{ij}$. Where Y_{ij} is the observation, μ is the general mean, T_i is the effect of hemp seeds and e_{ij} is the SE. The Tukey’s multiple range test was used to compare the mean difference at $P< 0.05$.

Results & Discussion

The results showed that using hemp seeds decreased ($P<0.05$) the potential of gas production, but the gas production rate was not influenced by supplementation. The amount of partitioning factor (PF), actually digested organic matter and microbial biomass of diet containing hemp seeds were higher ($P <0.05$) than the control diet (Table 1).

Adding hemp seeds to diet decreased the potential of gas production by camel’s foregut comparing with control diet. The low production of gas by hemp seeds may be due to the presence of anti-nutritional agents like tannins, hemp seeds contain tannin (Barani *et al.*, 2017). Tannin reduces methane production by eliminating rumen protozoa (Sliwinski *et al.*, 2002).

Table (1): Effect of supplementation of hemp seeds to the diet on fermentation and gas production parameters in dromedary camels

Item	CON	CON+HS	SEM	P-value
Potential of gas production (ml)	18.4[a]	14.2[b]	0.17	0.01
Gas production rate (ml/h)	0.027	0.031	0.01	0.25
Partitioning factor (mg/ml)	8.6[b]	13.5[a]	0.8	0.02
Microbial biomass (%)	182.7[b]	195.2[a]	4.1	0.03
Actually digested organic matter (mg)	201.3[b]	217.5[a]	0.3	0.02

SEM: Standard error of means, Means in a row with differing superscripts differ (*P<0.05*). Experimental diets included: control diet (CON) and diet containing 10% hemp seed (CON+HS)

One of the anti-nutrient factors of hemp seeds is phytic acid. Hemp seeds contain similar amount of phytic acid as soybean and sunflower (Cowieson *et al.,* 2004). Phytic acid decreases protein and amino acid degradability and increases endogenous nitrogen, amino acids, and minerals (Cowieson *et al.,* 2004). Hemp seeds containing tannins that reduce nitrogen digestibility, mineral uptake, weight gain and feed intake (Hessle *et al.,* 2008). Another cause of the reduction of gas production can also be related to fatty acids in hemp seeds.

Diets containing high amounts of fatty acids affect the digestion of the fiber in the rumen; especially high unsaturated fatty acids that have toxic effects on rumen flora and thus reduce fiber digestion (Alan, 2000). Scientists found that the oil of hemp seeds varied between 26.3% and 37.5%, and typically about 90% of hemp fatty acids are unsaturated (Callaway, 2004) that can influence the fermentation and gas production. According to current results, adding the hemp seeds increased partitioning factor, microbial biomass, and actually digestible organic matter. Researchers have argued that lower levels of PF suggest low levels of microbial protein synthesis (Sallam *et al.,* 2010). This means that a larger share of digested feed is spent on gas production relative to the synthesis of microbial protein (Sallam *et al.,* 2010). It may be due to the increased access of microorganisms to the nutrients and thus more gas production in these treatments. Therefore, its antimicrobial factors may prevent the use of nutrients produced in the microbial structure (Sallam *et al.,* 2010).

In the current study, it may be the toxicity of unsaturated polyunsaturated fatty acids in hemp seeds to bacteria and protozoa or severe decline of protozoa population, caused to increase of bacteria biomass and microbial protein that reaches duodenum (Bodas *et al.,* 2009). Hemp seeds supplementation had significantly (*P<0.05*) more *in vitro* digestibility by foregut bacteria of camel, after 24, 48 and 72 h incubation (Table 2).

The digestibility of diet containing hemp seeds increased due to increase of microorganism’s activity in comparison with the control treatment. The low content of NDF and ADF and the high proportion of non-fibrous carbohydrates improve digestion and fermentation (Tabaraki *et al.,* 2012). The studies indicated the oil of hemp seed is coated and will not interfere with rumen function, so it is expected to have no adverse effect on nutrient digestibility and even improve it (Pashaei *et al.,* 2014). Bodas *et al.* (2009) reported that the addition of some plants with effective matters stimulates bacteria activity and the digestibility of dry matter.The amount of essential amino acids of hemp seeds protein, especially arginine, is at a very favorable level that can protected by seed hull and can be used as a protein supplement. Hemp is also rich in minerals; especially iron that can prepare nutrients for better function of rumen microorganisms (Callaway, 2004).Technically, hemp seed typically contains about 25% protein, with considerable amounts of dietary fiber, vitamins, and minerals. The two main proteins in hemp seed are high-quality storage proteins, which are easily digested and contain nutritionally significant amounts of all essential amino acids. Hemp seeds protein have good amounts of the sulfur-containing amino acids methionine and cysteine, in addition to very high levels of arginine and glutamic acid (Odani & Odani, 1998). Another study on hemp seeds found that it can be an excellent source of rumen undegraded protein in cows and sheep (Mustafa *et al.,* 1999). It seems that the increased degradability by bacteria is probably due to the breakdown of some compounds in these oils by the microorganism. Part of digestibility increases may be due to the addition of soluble sugars and other nutrients in the feed (Sallam *et al.,* 2010). Results showed that medicinal plants containing secondary metabolites (hemp seeds containing phytic acid

Table (2): Effect of supplementation of hemp seeds to the diet on bacteria digestibility of dromedary camel's foregut in different times of incubation.

Item	CON	CON+HS	SEM	P-value
24 h				
Dry matter digestibility %	43.2 ^{b}	55.6 ^{a}	1.6	0.03
Organic matter digestibility %	24.2 ^{b}	29.6 ^{a}	2.1	0.01
Ammonia nitrogen (mg/dl)	13.2 ^{a}	11.4 ^{b}	0.3	0.02
pH	6.67	6.71	2.1	0.3
48 h				
Dry matter digestibility %	46.3 ^{b}	63.2 ^{a}	1.2	0.04
Organic matter digestibility %	36.4 ^{b}	41.4 ^{a}	0.1	0.01
Ammonia nitrogen (mg/dl)	12.2 ^{a}	11.1 ^{b}	0.4	0.02
pH	6.43	6.51	3.1	0.2
72 h				
Dry matter digestibility %	58.4 ^{b}	67.5 ^{a}	3.2	0.03
Organic matter digestibility %	46.3 ^{b}	52.3 ^{a}	2.2	0.02
Ammonia nitrogen (mg/dl)	14.2 ^{a}	10.7 ^{b}	1.1	0.05
pH	6.41	6.47	2.7	0.4

SEM: Standard error of means, means in a row with differing superscripts differ (*$P<0.05$*). Experimental diets included: control diet (CON) and diet containing 10% hemp seed (CON+HS)

and tannins), improve digestion in the rumen by altering the microbial population (Alexander *et al.*, 2008). The results of the current study showed the addition of hemp seeds decreased (*P<0.05*) ammonia nitrogen concentration compared to the control diet, but pH is not influenced by addition. Decreasing ammonia nitrogen concentration by addition hemp seeds can be due to proper utilization of nitrogen released in the rumen and simultaneous release of ammonia and energy for microbial protein production and therefore lower nitrogen excretion from the animal. Phytic acid of hemp seeds decreases protein and amino acid digestibility (Cowieson *et al.*, 2004), therefore decrease of amino acid digestibility decrease ammonia production in the rumen.It is also stated that tannins have a negative effect on the growth of proteolytic bacteria (Sallam *et al.*, 2010); therefore, the growth of these bacteria decreases the protozoa population of the rumen, which can also be attributed to the decrease in ammonia in this research. Bodas *et al.* (2009) observed the ineffectiveness of some plants on pH in the culture medium. Other reports also indicate the effect of fatty acids on ammonia nitrogen. Ammonia nitrogen production from amino acids in the rumen fluid was also reduced by fatty acids of the diet. The researchers found that the use of rapeseed oil and tallow reduced the concentration of ammonia nitrogen compared to the control group (Paya *et al.*, 2015). Using of fat in the diet caused to decrease in the protozoa number and the reduction of microbial nitrogen recovery. Therefore, fatty acids with the inhibitory effect on protozoa probably reduce ammonia nitrogen (Paya *et al.*, 2015).

Conclusions

In conclusion, using hemp seeds as feed additive caused a reduction in gas emission and concentration of ammonia nitrogen in the dromedary camel and increased nutrients digestibility by cellulolytic bacteria. Therefore it can be recommended in camel diets and will influence camel growth performance and decrease gas emissions in the environment.

Acknowledgments

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

All applicable institutional, national and international guidelines for the care and use of animals were followed.

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تأثير إضافة بذور القنب (*Cannavis sativa* L.) على معايير التخمير مختبريا والهضم باستخدام سائل الكرش للابل ذات السنام الواحد

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المستخلص: أجريت الدراسة الحالية لبحث تأثير بذور القنب على إنتاج الغاز الحيوي في المختبر والهضم في الإبل ذو السنام الواحد. غذيت إثنين من اناث الإبل (300 كجم) على عليقة تحتوي (60 قش القمح و 40 البرسيم) لمدة شهر واحد. ثم تم اخذ سائل الكرش من الإبل قبل وجبة الصباح. غذيت الابل على عليقتين الاولى (السيطرة) والعليقة الثانية تحتوي على 10٪ من بذور القنب. تم استخدام انابيب زجاجية سعة 100 مل تحتوي على 30 مل من سائل الكرش المخزن. قيست معايير إنتاج الغاز بواسطة النماذج الأسية وتم تحديد قابلية هضم بكتيريا الإبل. أوضحت النتائج أن إضافة بذور القنب قللت من احتمالية إنتاج الغاز، في حين لم يتأثر معدل إنتاج الغاز معنويا ($P > 0.05$) ، وكانت المواد العضوية المهضومة والكتلة الحيوية الميكروبية في العليقة التي يحتوي على بذور القنب أكثر معنويا ($P < 0.05$) مقارنة بالابل المغذاة على عليقة السيطرة ، واوضحت النتائج أن معاملة بذور القنب كان لديها أكبر قابلية للهضم عن طريق البكتيريا المحللة ، بعد حضانة 24 و 48 و 72 ساعة. واشارت نتائج الدراسة الى أن بذور القنب قد خفضت تركيز الأمونيا من النيتروجين مقارنة بمعاملة السيطرة . نستنتج من خلال ما تقدم ان إضافة بذور القنب الى عليقة الابل تسبب في الحد من انبعاث الغازات وإنتاج نتروجين الأمونيا وزيادة قابلية هضم البكتيريا الخلوية ؛ لذا يمكن استخدام بذور القنب في علائق الإبل ، اذ انها تؤثر على معدلات نمو الإبل وتقلل من انبعاثات الغازات في البيئة.

الكلمات المفتاحية: بذور القنب، انبعاث الغازات، قابلية الهضم في المختبر، الجمل العربي.