IMPACT OF THE ESSENTIAL OILS OF MARJORAM OR BASIL DIETARY SUPPLEMENTATION ON DEGRADABILITY, RUMINAL FERMENTATION AND TOTAL GAS PRODUCTION *IN-VITRO*

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SUMMARY

An *in-vitro* study was carried out to investigate the effect of using different levels of the essential oils of Marjoram (*Origanum majorana L.*) or Basil (*Ocimum basilicum L.*) as a natural feed additive on ruminal fermentation, total gas production, ammonia concentration, dry matter and cell wall contents (NDF and ADF) digestibility. Eleven experimental groups were used by *in-vitro* batch culture technique. The basal diet consisted of a total mixed ration (TMR) in all treatments, the experimental treatments were: 50% CFM, 50% alfalfa hay (control), control diet plus 5, 10, 15, 20 and 25 ml of Marjoram oil or Basil oils / kg DM for T1, T2, T3, T4, T5, T6, T7, T8, T9 and T10, respectively. No differences *($p>0.05$)* were observed between Marjoram or Basil oils in pH, NDFd and ADFd at all different levels compared with the control diet. Ammonia concentrations showed significant *($p<0.05$)* decreased for all the experimental diets compared with the control diet, except T6. Also, short chain fatty acid (SCFAs) contents were decreased for all treatments compared with control, except T8 and T9. The values cleared that Marjoram oils supplementation to diets showed a significant decrease ($p<0.05$) in total gas production versus control which recorded the highest value (128 ml) except T8 and T9. There was a significant increase ($p<0.05$) in dry matter degradability (DMd) between T4 and T9 compared with the control. Cell wall contents (NDF and ADF) digestibility values were not significant affected by oils supplementations. It could be concluded that supplementation of Marjoram or Basil oils to diets had negative influence on ruminal fermentation parameters (SCFAs, ammonia and total gas production). The Marjoram oil showed significantly superior DM degradability and reduction total gas production compared to the Basil oil.

Keywords: *Essential oils, Marjoram, Basil, digestibility, rumen fermentation and in-vitro.*

INTRODUCTION

Recently, the main goals of livestock production system are minimizing the usage of synthetic and medicinal chemistry versus non-antibiotic growth promoters or natural growth promoters like plant extracts as modifiers of rumen fermentation process (Matloup *et al.*, 2017; Khattab *et al.*, 2019 and 2020a). Essential oils are commonly used as feed additives to animals as therapeutics and/or prophylactic to some metabolic disorders as well as, improve feed efficiency. Several studies were carried out to investigate plant secondary metabolites such as flavonoids, polysaccharides, coumarin, alkaloids, saponins and essential oils and their usage as natural ruminal modifiers (Knapp *et al.*, 2014; Ishlak *et al.*, 2015; Cobellis *et al.*, 2016; Ali *et al.*, 2016; Abd El Tawab *et al.*, 2020 and 2021). Generally, the active components of essential oils revealed an antibacterial effect by deactivation of some microbial enzymes (El-Zaher *et al., 2020*). The antimicrobial properties of essential oils have been confirmed against a broad-spectrum of microorganisms such as bacteria, fungi and protozoa (Chao *et al.*, 2000; Abd El Tawab *et al.*, 2015 and 2019).

Many medical plants such as cinnamomum, rosemary, lemongrass, coriander, thyme, galangal and celery contained essential oils have been suggested on adjusting rumen fermentation and inhibition of deamination and methanogenesis, resulting in lower ammonia nitrogen, methane production and acetate, and in higher propionate and butyrate concentrations also, decreased ruminal bio-hydrogenation (Matloup *et al.*, 2017; Khattab *et al.*, 2016; 2017; 2020a; Abd El Tawab *et al.*, 2020 and 2021).

The essential oil of marjoram (*Origanum majorana L.*) contains thymol [(5-methyl-2-(1-methylethyl) phenol)], carvone, γ-terpinene, linalool and p-cymene depending on the origin and species of *Origanum* (Sivropoulou *et al.*, 1996 and Baser, 2002). Thymol is a monoterpene with strong antimicrobial activity against a wide range of Gram positive and negative bacteria (Walsh *et al.*, 2003 and Burt, 2004).

Basil (*Ocimum basilicum L.*) essential oil is determined based on the most common components (e.g. terpineol, linalool, limonene, methyl cinnamate, chavicol, eugenol and methyl eugenol) (Grayer *et al.*, 1996). Eugenol (4-allyl-2-methoxyphenol) is a phenolic monoterpene that has antimicrobial activity against gram-positive and gram-negative bacteria (Dorman and Deans, 2000 and Walsh *et al.*, 2003). Also, it has been able to reduce methane production or acetate, increase propionate production, and modify proteolysis, deamination or peptidolysis in the rumen (Calsamiglia, *et al.*, 2007 and Abd El Tawab *et al.*, 2021). Thus, the current study was aiming to investigate the effect of using different levels of Marjoram (*Origanum majorana L.*) or Basil (*Ocimum basilicum L.*) essential oils as a natural feed additive on ruminal fermentation, total gas production, ammonia nitrogen concentration, and dry matter, NDF and ADF degradability.

MATERIALS AND METHODS

Experimental diets:

In-vitro rumen fermentation technique incubation procedures were carried out as described by Abd El Tawab (2021). The basal diet consisted of a total mixed ration (TMR) in all treatments, the experimental treatments were: 50% CFM : 50% alfalfa hay (control), control diet plus 5 ml Marjoram oil / kg DM (T1), control diet plus 10 ml Marjoram oil / kg DM (T2), control diet plus 15 ml Marjoram oil / kg DM (T3), control diet plus 20 ml Marjoram oil / kg DM (T4), control diet plus 25 ml Marjoram oil / kg DM (T5), control diet plus 5 ml Basil oil / kg DM (T6), control diet plus 10 ml Basil oil / kg DM (T7), control diet plus 15 ml Basil oil / kg DM (T8), control diet plus 20 ml Basil oil / kg DM (T9), control diet plus 25 ml Basil oil / kg DM (T10). The chemical composition of CFM, alfalfa hay and experimental diets are presented in Table (1).

Table (1): Chemical composition of CFM, alfalfa hay and experimental diets (% DM basis).

Item	CFM	Alfalfa hay	Experimental diets
Dry matter	91.27	92.60	91.94
Organic matter	95.67	93.90	94.79
Crude protein	14.90	17.50	16.20
Ether extract	5.30	2.90	4.10
Non-fiber carbohydrate	54.14	29.62	41.88
Ash	4.33	6.10	5.22
Neutral detergent fiber	21.33	43.88	32.61
Acid detergent fiber	6.32	32.66	19.49

CFM: concentrate feed mixture; consisted of 57% yellow corn, 25% wheat bran, 15% soybean meal, 0.6% sodium bicarbonate, 1% limestone, 0.8% NaCl, 0.3% vitamins and 0.3% minerals.

Samples analysis:

After 24 h of incubation, gas production (GP), pH values and NH3-N concentration were analyzed according to Khattab and Abd El Tawab (2018). The amount of dry matter degradability (DMD) was determined according to AOAC (1995). Short chain fatty acids (SCFA) were calculated using the equation of Makkar (2005). Cell wall contents (NDF and ADF) were analyzed according to Van Soest *et al.* (1991). Gas production per gram DM, NDF and ADF were calculated according to Khattab *et al.* (2016).

Statistical analysis procedures:

Data were analyzed as a completely randomized design using GLM procedure of Statistical Analysis System (SAS, 2009), version 9.2. Significant differences between means of treatments were carried out by the Tukey's test, and the significance threshold was set at $p < 0.05$. The model was used.

$$Y_{ij} = \mu + T_i + e_{ij}.$$

Where Yij is the parameter under analysis of the ij flask of laboratory trails, µ is the overall mean, Ti is the effect due to treatment on the parameter under analysis, eij is the experimental error for ij on the observation,

RESULTS AND DISCUSSION

***Ruminal fermentation*:**

Data in Table (2) showed that the effect of Marjoram or Basil oils supplementation to diets on ruminal fermentation parameters. Generally, no differences *(p>0.05)* were observed between Marjoram or Basil oils in pH values at all different levels compared with control. While, there was insignificant *(P>0.05)* increased between treatments at high levels of Marjoram or Basil oils and control. pH values are an important index for investigating the inclusive essential oils impact on the microbial population and ruminal fermentation and subsequently the ruminal environment (Kumar *et al.,* 2013). The results in the present study affirmed the results from previous studies that found oregano (essential oil 1.4% or 1.58% of oregano leaf on DM) supplementation to lactating dairy cows' diets had no significant effect on rumen pH (Tekippe *et al.,* 2011 and Hristov *et al.,* 2013). Also, Evans and Martin (2000) found that ruminal pH was not affected at a lower dose of thyme oil (50, 100, 200 mg/ L) while, it was increased at 400 mg/ L (*in vitro)*. The increase in pH at high level of thyme oils could be related to the high levels of thymol which had a strong effect of rumen microbial fermentation and contribute to a reduction in methane, propionate, acetate and lactate concentrations. Castillejos *et al.* (2006) reported similar results after using different levels of various essential oils that contained some active components such as thymol, eugenol, limonene, guaiacol, ϒ-terpinene and vanillin and contributed to increased ruminal pH value reduced in ruminal fermentation and depression in TVFAs concentration. Moreover, Benchaar *et al.* (2007) reported an increase in the pH values with the supplementation of 400, 200, 400, 800 mg/L of cinnamon, thymol, carvacrol and eugenol respectively. Also, Busquet *et al.* (2006) suggested that the addition of 3g/L of eugenol and 0.3 and 3 mg/L of carvacrol contributed to the pH increase. Patra and Yu (2012) found a linear increase pH values when eucalyptus, clove, garlic, peppermint and origanum oils were added in doses of 0.10, 0.25, and 1 g/L. Kouazounde *et al.* (2015) found that the addition of essential oils from African basil (*Ocimum gratissimum*) to diets has increased the pH values of rumen fermenter liquid in the rumen simulation technique (RUSITEC).

Table (2): Effect of supplementing diet with different levels of marjoram or basil oils on some ruminal fermentation parameters.

Item	Control	Marjoram oil					Basil oil					*SEM*	*P value*
		T1	T2	T3	T4	T5	T6	T7	T8	T9	T10		
pH	6.75	6.74	6.84	6.82	6.84	6.85	6.76	6.78	6.81	6.84	6.85	0.028	0.099
Ammonia (mg/dl)	35.5[a]	30.7[bc]	27.5[cd]	26.7[cd]	25.7[d]	30.7[bc]	32.6[ab]	29.7[bcd]	27.8[cd]	25.6[d]	25.8[d]	0.582	<.0001
SCFA (mmol)	1.42[a]	1.25[c]	1.28[bc]	1.27[bc]	1.32[bc]	1.28[bc]	1.29[bc]	1.32[bc]	1.35[ab]	1.34[abc]	1.31[bc]	0.0088	<.0001

Control: 50% CFM, 50% alfalfa hay,T1: control diet +5 ml Marjoram oil / kg DM, T2: control diet + 10 ml Marjoram oil / kg DM, T3: control diet + 15 ml Marjoram oil / kg DM,T4: control diet + 20 ml Marjoram oil / kg DM, T5: control diet + 25 ml Marjoram oil / kg DM, T6: control diet + 5 ml Basil oil / kg DM, T7: control diet + 10 ml Basil oil / kg DM, T8: control diet + 15 ml Basil oil / kg DM, T9: control diet + 20 ml Basil oil / kg DM, T10: control diet + 25 ml Basil oil / kg DM.
SCFA; short chain fatty acids.
Different superscripts a, b, c and d at the same row differ significantly (p<0.05).
SEM: standard error of the means.

Ammonia concentrations showed significant (p<0.05) decreased for all the experimental diets compared with the control diet, except T6. the chemical components of essential oils had a variable impact on ruminal nitrogen metabolism in different studies. A decreased in ammonia-N concentration is suggesting the potentiality of Marjoram or Basil oils for inhibiting deamination. These results were consistent with McIntosh *et al.* (2000) who noted that essential oils during an i*n-vitro* study prevented deamination of amino acids in the rumen measured by 25%, these potential related to inhibiting rumen microbe's attachment to feed particles (Wallace *et al.,* 2002). Also, Castillejos *et al.* (2005) reported that the microorganism's species and population were greatly affected by essential oils as the same of those in antibiotics. They also reported that essential oils had inhibited hyper-ammonia producing (HAP) bacteria such as *Peptostreptecoccus anaerobius* and *Clostridium sticklandii* associated with a reduction of ammonia production, while the other hyper-ammonia producing species such as *Clostridium aminophilum* was less sensitive affected (McEwan *et al.,* 2002 and McIntosh *et al.,* 2003).

Hyper-ammonia producing bacteria are present in low numbers in the rumen fluid (less than 0.01 of the rumen bacterial population) but, it has a very high deamination activity (Russell *et al.,* 1988). Collectively, results of the studies by McIntosh *et al.* (2003), Wallace (2004) and Newbold *et al.* (2004) reported that essential oils produced an inhibitory influence on Hyper-ammonia producing bacteria contributing to lowered ruminal protein metabolism and amino acids degradation (deamination). Differences in the effects of essential oils supplementation to diets on NH3-N between *in vivo* or *in vitro* studies could be adequate in part by the capability of rumen microbial populations to degrade and / or adapt essential oils components. (Benchaar and Greathead, 2011). Busquet *et al.* (2006) found that the carvacrol when added a dose of 3 g/L decreased the concentration of ammonia nitrogen during *in-vitro* batch cultures.

Short chain fatty acid (SCFAs) contents were significantly decreased for all treatments compared with control, except T8 and T9, the control recorded the highest value (1.42 mmol). Reduction of SCFAs in Marjoram or Basil oils treatment could be a great index of simultaneous with methane reduction in the rumen tract (Busquet *et al.,* 2006). The change in molar proportions of TVFs led to a lack of a change TVFs concentration, decreased methane production and decreased NH3–N concentration. However, essential oils supplementation to diets leads to a reduction in TVFAs production and was viewed as nutritionally unfavorable (Khattab *et al.,* 2020a). Kouazounde *et al.* (2015) found that TVFAs production was linearly decreased by African basil *(Ocimum gratissimum)* essential oils supplementation to diets with a shift in VFAs profile towards more acetate and butyrate and less propionate. While, other studies revealed that certain essential oils and their components have the same manner as the antibiotics to shift molar proportions of VFAs (i.e. increased propionate and decreased acetate proportions) (McGuffey *et al.,* 2001). Also, Mohammed *et al.* (2004) found increased propionate and decreased acetate proportions with encapsulated cyclodextrin horseradish in both *in-vivo* and *in-vitro* studies*.* The SCFAs concentration and the proportion of propionate to acetate were decreased, while acetate to propionate ratio was increased when thymol (0.4 g/L) was used (Evans and Martin, 2000). Also, Castillejos *et al.* (2006) suggested the similar results for limonene, thymol, guaiacol and eugenol components using doses of more than 5g/L. Moreover, Kamalak *et al.* (2011) reported that thymol supplementation up to 0.2 g/L had no significant effect on molar propionate, acetate or butyrate proportions.

Gas Production:

In-vitro rumen gas production measurements of experimental treatments supplemented with different levels of Marjoram or Basil oils are illustrated in Table (3). The values cleared that Marjoram oils supplementation to diets showed a significant decrease ($p<0.05$) in total gas production (TGP) compared with control treatment which, recorded the highest value (128 ml) while, it was insignificantly increased ($P>0.05$) versus T8 and T9 treatments (122 and 121 ml), respectively. Also, the current results revealed that gas production per each gram of NDF recorded the same trend of total gas production (Table 3). Gas production per each gram of DM and ADF showed a significant decrease ($P<0.05$) in essential oils treatments compared with control treatment (346 and 1763 ml, respectively) while, it was insignificantly increased ($p>0.05$) versus T7, T8 and T9 treatments. Supplementation of essential oils have caused positively affect gas production and modified rumen fermentation in various *in-vitro in-vivo* studies (Macheboeuf *et al.,* 2008; Patra and Yu, 2012; Lin *et al.,* 2012; Cobellis *et al.,* 2016 and Khattab *et al.,* 2020a). These findings were synchronized with Benchaar *et al.* (2007) who recorded a reduction in gas emission when several types of essential oils such as thymol (0.2 g/L), carvacrol (0.4 g/L) and eugenol (0.8 g/L) were tested with an in-vitro batch fermentation system. Also, Kouazounde *et al.* (2015) reported that the addition of essential oils from African basil (*Ocimum gratissimum*) to diets decreased the total gas production and methane emission in the rumen simulation technique (RUSITEC). Oh *et al.* (1967) suggested an inhibition of rumen microbial activity based on the decrease in total gas production when a 0.12 ml/L dose of terpenoid essential oils from Douglas-fir needles leaves (limonene, α-pinene, myrcene, β-pinene, terpinolene, A3-carene, and cz amphene) was used an in-vitro study. Rezaei and Pour (2012) noted that the total gas production was decreased with the addition of thyme methanolic extracts. Also, Chaudhry and Khan (2012) found that by using an *in-vitro* gas production technique, five curry spices such as cumin, turmeric, coriander, cinnamon and clove as act a non-antibiotic growth promoter and contribute to killing *methanogenic bacteria* which lead to a reduction of methane emission by 40%. Nagy and Tengerdy (1968) suggested that essential oils extracted from Sagebrush (*Artemesia tridentata*) markedly caused inhibition of rumen bacterial activity as gas production during *in vitro* study. Moreover, the degree of inhibition depended on the chemical composition of the essential oils compound added.

These compounds are divided into particularly monoterpene alcohols, aldehydes and oxygenated monoterpenes, that strongly inhibited the metabolism and growth of rumen microbes. Whereas, monoterpene hydrocarbons stimulated or slightly inhibited the activity of rumen microbes (Benchaar *et al.,* 2008). Kim *et al.* (2012) evaluated that the herbal plant extracts (*Mandarin orange, Lonicera japonica; Honeysuckle; Artemisia princeps var. Orientalis; Garlic, Allium cepa; Ginger, Citrus unshiu; Onion, Zingiber officinale; Wormwood, Allium sativum for. Pekinense*) were shown to have properties to decrease methanogens bacteria, methane production and acetate to propionate ratios and increase fibrolytic bacteria species. It is well-known that the cell wall contents ADF were negatively correlated with gas production which tends to reduce the microbial activity (Khattab and Abd EL Tawab, 2018).

Table (3): Effect of supplementing diet with different levels of marjoram or basil oils on ruminal gas production (ml).

Item	Control	Marjoram oil					Basil oil					*SEM*	*P value*
		T1	T2	T3	T4	T5	T6	T7	T8	T9	T10		
TGP	128[a]	113[c]	116[bc]	115[bc]	119[bc]	116[bc]	116[bc]	120[bc]	122[ab]	121[abc]	118[bc]	0.810	0.0001
GP/gram DM	346[a]	305[b]	312[b]	307[b]	319[b]	314[b]	314[b]	322[ab]	329[ab]	326[ab]	319[b]	2.266	0.0003
GP/gram NDF	1057[a]	934[b]	950[b]	952[b]	981[b]	959[b]	960[b]	985[b]	1005[ab]	995[ab]	977[b]	6.694	0.0003
GP/gram ADF	1763[a]	1561[b]	1586[b]	1591[b]	1637[b]	1602[b]	1604[b]	1644[ab]	1677[ab]	1660[ab]	1631[b]	11.054	0.0005

Control: 50% CFM, 50% alfalfa hay,T1: control diet +5 ml Marjoram oil / kg DM, T2: control diet + 10 ml Marjoram oil / kg DM, T3: control diet + 15 ml Marjoram oil / kg DM,T4: control diet + 20 ml Marjoram oil / kg DM, T5: control diet + 25 ml Marjoram oil / kg DM, T6: control diet + 5 ml Basil oil / kg DM, T7: control diet + 10 ml Basil oil / kg DM, T8: control diet + 15 ml Basil oil / kg DM, T9: control diet + 20 ml Basil oil / kg DM, T10: control diet + 25 ml Basil oil / kg DM.
Total GP; total gas production after 24hours.
Different superscripts a, b, c and d at the same row differ significantly ($p<0.05$).
SEM: standard error of the means.

Nutrients degradability:

In-vitro nutrients degradability is presented in Table (4) revealed a significant increase (*$P<0.05$*) in

Table (4): Effect of supplementing diet with different levels of marjoram or basil oils on ruminal nutrients degradability (%).

Item	control	Marjoram oil					Basil oil					*SEM*	*P value*
		T1	T2	T3	T4	T5	T6	T7	T8	T9	T10		
DMd	37.6[c]	38.5[c]	39.9[abc]	39.2[bc]	44.0[a]	40.1[abc]	40.1[abc]	40.5[abc]	40.8[abc]	43.9[ab]	41.4[abc]	0.411	0.0014
NDFd	36.1	35.5	36.3	36.4	36.6	35.0	35.0	36.5	34.8	35.2	34.2	0.454	0.9902
ADFd	31.7	27.4	29.1	31.5	32.1	28.0	28.8	27.2	26.6	26.0	31.5	0.569	0.1296

Control: 50% CFM, 50% alfalfa hay,T1: control diet +5 ml Marjoram oil / kg DM, T2: control diet + 10 ml Marjoram oil / kg DM, T3: control diet + 15 ml Marjoram oil / kg DM,T4: control diet + 20 ml Marjoram oil / kg DM, T5: control diet + 25 ml Marjoram oil / kg DM, T6: control diet + 5 ml Basil oil / kg DM, T7: control diet + 10 ml Basil oil / kg DM, T8: control diet + 15 ml Basil oil / kg DM, T9: control diet + 20 ml Basil oil / kg DM, T10: control diet + 25 ml Basil oil / kg DM.
DMd: dry matter disappearance; NDFd: natural detergent fiber disappearance; ADFd: acid detergent fiber disappearance.
Different superscripts a, b, c and d at the same row differ significantly ($p<0.05$).
SEM: standard error of the means.

dry matter degradability (DMd) between T4 and T9 being (44 and 43.9 %, respectively) compared with control and T1 which recorded the lowest values being (37.6 and 38.5 %, respectively). The enhanced dry matter digestibility with the addition of Marjoram or Basil oils confirmed the beneficial effect of their active components. It seems credible to indicate that the concentrations of active components in

Marjoram or Basil oils were below the sill levels at which nutrient digestion is inhibited (Khattab *et al.*, 2016; 2017; 2020a and 2020 b). The essential oils contain several secondary metabolites and hydrocarbons (e.g., Terpineol, linalool, Limonene, methyl cinnamate, thymol and limonene), with different dose-dependent antimicrobial activities against some ruminal protozoa and bacteria species (Benchaar *et al.,* 2008). The improved digestion confirmed that the dose of Marjoram or Basil oils were lower than that dose which destroys the activities of ruminal microorganisms (Walsh *et al.,* 2003). These results were in agreements with Khattab *et al.* (2020b) who found that the dry matter digestibility increased by thyme supplementation to ewes' diets (20g/h/d).

While, cell wall contents degradability (NDFd and ADFd) results appeared non-significant ($p>0.05$) variances between control and other treatments. These findings were in agreement with Patra (2011) who found the essential oils have no negative effect on fiber degradation (NDFd and ADFd). Also, Khattab *et al.* (2020a) showed the similar results when they used celery and thyme in an *in vitro* study. Kali (2017) found that NDFd and ADFd were not affected by experimental treatment when used a pellet containing Stay Strong (a commercially available blend of essential oils) in lactating dairy cows. Also, Castillejos *et al.* (2006) suggested no negative effect on activity and growth of the major cellulolytic bacterial population by adding 0.005, 0.05, and 0.5 g/L of eugenol in a continuous-culture fermenter, and reported no effects on NDF, and ADF digestibility.

CONCLUSION

Based on the conditions of the present *in-vitro* study. It could be concluded that supplementation of Marjoram or Basil oils (a natural feed additive) to diets contributed to a negative influence on ruminal fermentation parameters (SCFAs, ammonia-N and total gas production), while dry matter degradability was increased at level 20 ml / kg DM. The Marjoram oil showed superior DM degradability and reduction in total gas production compared to the Basil oil. Recently, the *in-vivo* study is carrying out to investigate more topics such as the effect of those essential oils (Marjoram or Basil) on the lactational performance of dairy goats.

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تأثير اضافة الزيوت العطرية للبردقوش او الريحان للعلائق على عملية الهضم وتخمرات الكرش وانتاج الغاز الكلي معمليا

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اجريت دراسة معملية لدراسة تأثير استخدام مستويات مختلفة من الزيوت العطرية من البردقوش (*Origanum majorana L.*) أو الريحان (*Ocimum basilicum L.*) كإضافة طبيعية على تخمرات الكرش وإنتاج الغاز الكلي وتركيز الأمونيا وعلي هضم كلا من المادة الجافة ومكونات جدر الخلايا (NDF , ADF). تم استخدام إحدى عشرة مجموعة تجريبية بتقنية محاكاة هضم الكرش معمليا. اشتملت العليقة الاساسية علي مخاليط علفية (TMR) في جميع المعاملات ، وكانت المعاملات التجريبية: 50٪ مخلوط علف مركز و 50٪ دريس البرسيم (المجموعة المقارنة) ، والمجموعة المقارنة مضاف اليها 5 ، 10 ، 15 ، 20 ، 25 مل من زيت البردقوش او زيوت الريحان / كجم مادة جافة لـ T1 و T2 و T3 و T4 و T5 و T6 و T7 و T8 و T9 و T10 على التوالي. لم يلاحظ وجود فروق معنوية (P>0.05) بين زيوت البردقوش أو الريحان في pH وهضم او تحلل NDF وال ADF في جميع المستويات المختلفة مقارنة بالمجموعة المقارنة. أظهرت تراكيز الأمونيا انخفاضاً معنوياً (P<0.05) لجميع المعاملات التجريبية مقارنة بمجموعة المقارنة باستثناء T6.كما انخفضت محتويات الأحماض الدهنية قصيرة السلسلة (SCFAs) لجميع المعاملات مقارنة بالمجموعة المقارنة ، باستثناء T8 و . T9أوضحت النتائج أن إضافة زيوت البردقوش للعلائق أظهر انخفاضًا معنويًا (P<0.05) في إجمالي إنتاج الغاز مقارنة بالمجموعة المقارنة حيث سجلت أعلى قيمة (128 مل) باستثناء T8 و T9 .كانت هناك زيادة معنوية (P<0.05) في هضم المادة الجافة (DMd) بين T4 و T9 مقارنة بالمجموعة المقارنة. لم تتأثر محتويات هضم جدر الخلايا معنويا باضافة الزيوت. يمكن الاستنتاج أن إضافة زيت البردقوش أو الريحان إلى العلائق كان له تأثير سلبي على معدلات التخمر في الكرش(الأحماض الدهنية قصيرة السلسلة ، الأمونيا وإجمالي إنتاج الغاز). كما أظهر اضافة زيت البردقوش تفوقًا ملحوظًا في هضم المادة الجافة وخفض إنتاج الغاز الكلي مقارنة بزيت الريحان.