Effect addition of the peppermint oil to barley straw in some characteristics of fermentation in the rumen, in vitro digestibility and metabolic energy

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Abstract
This study aimed to investigate the influence of adding different concentrations of peppermint oil to barley straw in some fermented rumen features, in vitro digestibility and energy of metabolism. The results showed a significant decrease (p<0.01) in the pH of all treatments except T4, during all incubation period in comparison with the control treatment. The results also presented a significant increase (p<0.01) in the concentration of the nitrogen of ammonia in treatments of T3 and T4 and during every part of incubation periods in comparing with the control treatment. Additionally, a significant decrease (p<0.01) was discovered in the in vitro dry matter, digestibility organic substances and metabolic energy compared with the treatment of control.

Keywords: Peppermint oil, Gas production, Barley straw, some characteristics of rumen fermentation, in vitro digestibility

1. Introduction
The issue of the application of antibiotics in animal feeding has received considerable critical attention because of the possible emergence of resistant bacteria through the cultural fodders and raise the percentage of methane production [1,2]. Consequently, the natural components particularly the essential or volatile oils are an increasingly important and attractive area to be used as natural alternatives to the antibiotics in animal feeding. This is due to their significant activities against a wide range of microorganisms of the rumen [3]. A considerable amount of literature has been published on the essential or volatile oils. These studies proved that these oils have Inhibiting effect on the microorganisms exist in the rumen [4] in addition to reducing of methane production in ruminants [5].

As a result of the fermentation process of organic substances inside rumen, several gases are produced. The quantity of gas produced in ruminants differs due to impact numerous factors such as the pH value of rumen, microorganisms strains, the concentrations of acetic acid and probiotic, the composition of the fodder and the number of concentrated fodders delivered to the animals [6]. Ruminant animals produce different gasses, which represent 17-37 % of total gas production [7]. Additionally, the methane represents approximately 8-12% loss of total feed energy and 11-13% of digested energy [8]. Therefore, it has been a fundamental concern to preserve feed energy through dropping the formation of methane gas production by utilizing some diverse feed additives and other additions such as the antibiotics [9]. However, some of these supplements such as the antibiotics are having a serious limitation because of its harmful influence on animal health and microorganisms that live in the rumen. As well as, they can lead to the emergence of bacteria that are resistant to antibiotics in addition to possibility deposition of them in milk and meat consumed [10]. As a consequence of these reasons, researchers and animal nutritionists have inspired to examine new alternatives such as plant extracts and essential oils of medicinal plants such as the mint oil for reduction of methane production in the rumen [11]. The aim of this research project has therefore been to obtain the best.
the best ratios of peppermint oil added to the mixed fodder (concentrated feed + coarse feed) in its effect on the production of methane gas and some properties of fermentation in the rumen (acidic function, ammonia’s nitrogen concentration, digestibility factor, and the amount of metabolic energy) after different periods of incubation.

2. Material and methods

The study was accomplished in the Laboratory Nutrition belongs to the Agriculture College / University of Baghdad for the period started from 2/5/2017 to 2/6/2017. Several concentrations of mint oil (0, 70, 140 and 280 µl/kg) were added into the fodder mixture, which was constructed of the barley hay (80%) and the concentrated fodder (20%). This was to study the influence of fodder type and the addition of peppermint oil on the pattern of rumen fermentation and laboratory digestion factor for dry matter, organic matter and metabolic energy in the fodder. Tables 1 and 2 reveals the chemical composition of the concentrated, barley hay and cultural fodders in which used in this study with peppermint oil.

Table 1. The chemical composition of the ingredients of the concentrated and coarse fodders in cultural fodders used in this study

| Fodder substances | Dry matter (%) | Organic Material (%) | Ash (%) | Crude Protein (%) | Crude fibre (%) | Ether Extract (%) | The extract free of Nitrogen (%) | Metabolic energy (Megajoule/kg dry matter) * |
|-------------------|----------------|----------------------|---------|------------------|----------------|------------------|--------------------------------|----------------------------------|
| Barley            | 89.54          | 84.87                | 4.67    | 11.70            | 7.56           | 2.08             | 63.53                          | 11.32                             |
| Yellow corn       | 89.12          | 82.14                | 6.98    | 9.25             | 2.91           | 4.75             | 65.23                          | 11.86                             |
| Soybean meal      | 89.23          | 84.21                | 5.02    | 44.11            | 6.55           | 2.30             | 31.25                          | 10.70                             |
| Wheat bran        | 89.65          | 83.85                | 5.80    | 15.06            | 12.34          | 4.77             | 51.68                          | 11.13                             |
| Barley hay        | 88.46          | 82.13                | 6.33    | 5.01             | 34.15          | 1.52             | 41.45                          | 8.86                              |

* The metabolic energy (Mega joule/kg dry matter) = 0.012 x crude protein + 0.031 x crude fat + 0.005 x crude fiber + 0.014 x dissolved carbohydrates [12].

Table 2. The chemical composition of the experimental treatments involved the peppermint oil at various concentration (0, 70,140 and 280 µl/kg of dry matter)

| Nutrients                  | T1    | T2    | T3    | T4    |
|---------------------------|-------|-------|-------|-------|
| Dry matter (%)            | 90.63 | 90.48 | 90.03 | 90.43 |
| Organic matter (%)        | 84.25 | 84.07 | 83.67 | 84.06 |
| Ash (%)                   | 6.38  | 6.41  | 6.36  | 6.37  |
| Crude protein (%)         | 5.07  | 5.05  | 5.04  | 5.02  |
| Crude fiber (%)           | 34.13 | 34.18 | 34.14 | 34.12 |
| Ether extract (%)         | 1.55  | 1.57  | 1.53  | 1.58  |
| The extract free of Nitrogen (%) | 43.50 | 43.27 | 42.96 | 43.34 |
| Metabolic energy (Mega joule/kg of dry matter) | 8.88 | 8.85 | 8.80 | 8.86 |

T1: control treatment (without additions); T2, T3 and T4 are treatments with peppermint oil at a concentration of 70, 140 and 280 µl/kg of dry matter respectively.

2.1 Measurement of the acidy function

The pH value of the whole samples was measured directly after incubation periods. The measurement was conducted using the Digital PH Meter PH-009 (Lucky stone CO., LTD. / Taiwan).

2.2 Measurement of the nitrogen concentration of ammonia

Samples of rumen fluid (10 ml) were gathered after each incubation period from all experimental fodders and then stored in the freezer for later chemical analysis. This analysis was executed after melting through centrifuging them at a speed of 3000 rpm until earning pure yellow liquid contamination free. Afterwards, 0.5 ml of the rumen liquid was drawn and combined with 0.5 g of magnesium oxide. The mixed sample was then placed in the distillation apparatus (Micro-Kjeldahl)
and then wipe with 0.05% of the hydrochloric acid to acquire a reading, which was operated according to the following equation to reveal the nitrogen of ammonia content in the rumen fluid [13].

\[
\text{NH}_3\text{-N} \% = \frac{\text{Amount of Titrated acid} - \text{Blank} \times 0.05 \times 0.014 \times 100}{\text{Sample size (ml)}}
\]

### 2.3 The laboratory digestibility coefficient of dry material (IVDMD) and the organic material (IVOMD)

The laboratory digestibility factor for the dry and organic matter was estimated according to a previous method [14]. The peppermint oil was added in various concentrations (0, 70, 140, 280 l / kg dry matter) to the fodder mixture (80% barley hay + 20% concentrated fodder) and then mixed thoroughly. A 0.5 g of the mixture was placed inside the digestion tubes with adding 50 ml of mixture that consists of 10 ml of filtered rumen liquid collected from newly slaughtered ram and 40 ml of artificial saliva prepared at the same time. The carbon dioxide was added twice daily to create anaerobic conditions during the period of laboratory incubation and the modification of the acid function. The tubes were then placed in a water bath at a temperature of 39 °C for 48 h with shaking twice a day. At the end of the incubation time, the tubes cooled by placing them in cold water with continuous stirring, filtered and then added to the precipitate 50 ml of the mixture consisting of 0.2 g Pepsin enzyme that was dissolved in 100 ml of standard hydrochloric acid 0.1 N. The tubes are shaken well to break down the undigested parts and to kill the microorganisms. Later, they were again incubated at the same temperature for 48 hours, then the samples were filtered and the sediment was taken, which represents the products of the microbial and enzymatic digestion stages. After that the sediment was dried at a temperature of 105 °C for 24 h in order to calculate the percentage of the digestibility coefficient of dry matter, subsequently, samples were placed in the oven at a temperature of 600 °C for 3 h for estimation the percentage of the ash. The following equations were employed to calculate the laboratory digestibility coefficient of dry matter and organic material.

\[
\text{Digestion coefficient of dry matter} = \frac{\text{Sample weight} - \text{the weight of undigested dry matter} - \text{the dry matter of the blank}}{\text{Sample weight}} \times 100
\]

\[
\text{Digestion coefficient of organic matter} = \frac{\text{Weight of the organic matter in the sample} - \text{the weight of undigested organic matter} - \text{Weight of the organic matter in the blank}}{\text{Weight of the organic matter in the sample}} \times 100
\]

### 2.4 Estimation amount of metabolic energy

The metabolic energy (ME) was calculated using the following formula: ME = 0.15 x digestibility factor (%) of the dry matter [11].

### 2.5 The statistical analysis

The entire experimental data were statistically analyzed according to Completely Randomized Design (C.R.D) in four replicates. The Duncan test was employed to compare the averages of treatments [15]. Additionally, the SAS statistical program was utilized according to the following mathematical pattern:

\[
Y_{ij} = \mu + ti + \delta j
\]

\(Y_{ij}\) = The value of the observed studied
\(\mu\) = The general mean of the examined characteristics
\(ti\) = The treatment effect i (Four levels of addition)
\(\delta j\) = The random error, which is distributed naturally with an average of zero and varies equally to \(\delta j\) e.

### 3. Results and discussion

#### 3.1 Measurement of the acidy function

Effect of different concentrations of peppermint oil added into barley hay on pH value was examined. In Table (3) there is a clear trend of occurring a significant differentiation (p<0.01) in pH values. The highest value of pH after 12 and 24 h of
incubation was found in treatment T4 reached 7.15 and 7.22 respectively, followed by control treatment T1 that was 6.80 and 6.61 respectively. Treatments T3 and T2 were in the third and last rank among treatments with pH values reached 6.52, 6.57 and 6.07, 6.22 respectively. Similarly, in the same table high significant differences (p<0.01) in pH values were identified in barley hay that mixed with different levels of the peppermint oil after 48 and 72 h of incubation. Treatments T2 and T3 recorded a significant drop (p<0.01) achieved the averages 6.17, 675 and 6.35, 6.22 respectively. These treatments did not differ significantly with the treatment of control reached 6.70 and 6.82 respectively. Conversely, treatment T4 caused a highly significant raise (p<0.01) reached 7.18 and 7.25 respectively. However, the findings of the current study do not support previous research [17].

There are several possible explanations for this result, for instance, the addition of peppermint oils to fodders keeps pH values low. This is due to the occurrence of some inhibition of the rumen microorganisms that analyze the protein. Nevertheless, the pH remains within the normal range as a result of the increased decomposition of ester bonds and increase the concentration of fatty acids in the rumen leading to the availability of optimal conditions for the rumen microorganisms to digest both fibre and protein [18].

### Table 3. The impact of adding peppermint oil on the value of pH in the laboratory

| The traits studied | pH values          | The incubation period (hour) |
|-------------------|--------------------|-----------------------------|
| Treatments        | 12         | 24     | 48     | 72      |
| T1                | 0.095±6.80 B    | 0.091±6.61 B | 0.091±6.70 B | 0.095±6.82 B |
| T2                | 0.015±6.07 C    | 0.086±6.22 B | 0.016±6.17 C | 0.073±6.75 B |
| T3                | 0.043±6.52 B    | 0.033±6.57 B | 0.224±6.35 B | 0.010±6.22 C |
| T4                | 0.037±7.15 A    | 0.066±7.22 A | 0.026±7.18 A | 0.064±7.25 A |

T1: control treatment (without additions); T2, T3 and T4 are treatments with peppermint oil at a concentration of 70, 140 and 280 µl/kg of dry matter respectively.

** The presence of highly significant differences at the level of 0.01. The diverse letters in the same column mean there is a significant differentiation.

### 3.2 Measurement the nitrogen concentration of ammonia

The average scores of the concentration of ammonia’s nitrogen were compared to investigate effect adding a various level of the peppermint oil into barley hay during different incubation periods on these concentrations. The results, as shown in Table 4, indicate clearly to exist a highly significant difference (p<0.01) in treatments T3 and T4 after 12 and 24 h of incubation periods that were 27.82, 28.79 and 27.17, 28.68 mg/100 ml respectively. In contrast, in the same periods of laboratory incubation the nitrogen concentrations were decreased significantly (p<0.01) in treatments T2 (23.65 and 25.38 mg/100ml) and treatment of control (22.47 and 23.71 mg/100ml). Nevertheless, the results of the current study do not agree with the former investigation [19]. A possible explanation for this might be that the addition of peppermint oils to coarse fodders served to inhibit the bacteria producing ammonia, which had a clear consequence on eradicating the active amine group from the proteins and a decrease in the concentration of ammonia’s nitrogen in the rumen liquid.

Likewise, the results, presented in Table 4, demonstrated also the nitrogen concentration of ammonia after 48 and 72 h of incubation where the concentrations were raised significantly (p<0.01) in treatments T3 (29.71 and 29.93 mg/100ml) and T4 (29.09 and 29.27 mg/100ml). Furthermore, it was noted from same table that the effect in treatments T1 and T2 had a similar effect on the decrease in the concentration of ammonia’s nitrogen, as their rates were recorded at 24.44, 24.61 and 25.22, 26.35 mg / 100 ml respectively. These findings further support the results were found previously [20]. A possible explanation for these results may be due to the peppermint oils affected the nitrogen concentration of ammonia that had decreased in samples drawn from the rumen liquid. These concentrations were the lowest value of the T3 and T4 treatments, which were used for the highest growth of the microbial protein in the rumen.
Table 4. The effect of adding peppermint oil on the nitrogen concentration of ammonia

| The traits studied | The concentrations of ammonia’s nitrogen (mg/100 ml) |
|--------------------|-----------------------------------------------------|
| Treatments         | 12 | 24 | 48 | 72                 |
| T1                 | 0.017± 22.47 C | 0.020± 23.71 C | 0.021± 24.44 C | 0.018± 24.61 C |
| T2                 | 0.013± 23.65 B | 0.012± 25.38 B | 0.014± 25.22 B | 0.025± 26.35 B |
| T3                 | 0.010± 27.82 A | 0.018± 28.79 A | 0.034± 29.71 A | 0.034± 29.93 A |
| T4                 | 0.013± 27.17 A | 0.008± 28.68 A | 0.031± 29.09 A | 0.033± 29.27 A |

Significance Level

** The presence of highly significant differences at the level of 0.01. The diverse letters in the same column mean there is a significant differentiation.

3.3 The laboratory digestibility coefficient of dry and organic materials, and metabolic energy

The results in Table 5 are quite revealing in several ways. A highly significant boost (p<0.01) were discovered in the digestibility coefficient of dry substance (IVDMD) in barley hay in which different levels of peppermint oil mixed with by increasing of this digestibility coefficient in control treatment T1 into 55.71% comparing with treatments T2 (52.32%) and T3 (51.05%) respectively. However, these results did agree with the finding of the previous study [21]. Possibly the reason for this is that the addition of peppermint oils to a barley hay fodder did not affect to the activity of the microbes of the rumen liquid nor its number and its enzymatic efficacy [22].

Regarding the laboratory digestibility coefficient of the organic material (IVOMD), a significant decrease (p<0.01) was recorded and the lowest percentage was found in treatment T3 (52.67%), followed by treatment T2 (53.12%) and treatment T4 (53.78%) comparing to 56.45% in the treatment of control. These findings further support the results found previously [17]. A possible explanation to these findings is adding the different levels of the peppermint oil into fodder had not influenced on the activity of the microorganisms living rumen and method of the fermentation process in the rumen. Although the energies of metabolism were declined to 7.84, 7.65 and 7.89 (Megajoule/ kg of dry matter) in treatments T2, T3 and T4 respectively compared to 8.35 (Megajoule/ kg of dry matter) in control treatment T1, they did not differ significantly. However, these results differ from a previously published study [23]. It is challenging to explain these results, but it might be related to unsuitable levels of peppermint that added into fodder of barley hay in digestion processes, which cause a drop in the digestibility coefficient of dry and organic materials [24,25] as a result of inhibition the microorganism activity in samples collected from rumen liquid.

Table 5. The effect of adding peppermint oil on the laboratory digestibility coefficient of dry and organic materials, and metabolic energy

| The traits studied | The laboratory digestibility coefficient of dry material (IVDMD) % | The laboratory digestibility coefficient of organic material (IVOMD) % | The metabolic energy (Megajoule/ kg dry matter) |
|--------------------|---------------------------------------------------------------|---------------------------------------------------------------|-----------------------------------------------|
| Treatments         |                                                              |                                                              |                                               |
| T1                 | 0.023±55.71 A                                                | 0.022±56.45 A                                                | 0.032±8.35 A                                 |
| T2                 | 0.056±52.32 B                                                | 0.033±53.12 B                                                | 0.074±7.84 B                                 |
| T3                 | 0.044±51.05 B                                                | 0.018±52.67 B                                                | 0.057±7.65 B                                 |
| T4                 | 0.029±52.63 AB                                               | 0.016±53.78 B                                                | 0.031±7.89 AB                                 |

Significance Level

**

T1: control treatment (without additions); T2, T3 and T4 are treatments with peppermint oil at a concentration of 70, 140 and 280 µl/kg of dry matter respectively.
** The presence of highly significant differences at the level of 0.01. The varied letters in the same column mean there is a significant differentiation.

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