Research Article

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# Effect of Addition of Urea to The Barley Hay For Production of Gases In Vitro

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**Abstract**

The object of this study were determined the effect of adding urea (0,2%) and molasses (4%) to barley and straw on in vitro gas and methane production, in vitro fermentation characteristic (pH and ammonia nitrogen concentration) and in vitro digestibility, dry matter, organic matter and metabolizable energy, the samples incubated in 39°c water path for 24,48,72 and 96 h., the results indicated that there were significant decrease ($p<0.01$) in total gas and methane production after 48,72 and 96 h incubation periods in barley straw with 2% urea compared with control (without urea), while 24 h. total gas and methane production increased significant($p<0.01$) in straw with 2% urea compared with control. However, metabolizable energy, organic matter digestibility, short chain fatty acids and metabolizable energy for lactation which determine by total gas production after 24 h. incubation increased significantly ($p<0.01$) in straw with 2% urea compared with no adding urea. The result showed no effects of treatments on pH in all incubation periods except 48h. were pH increased significantly ($p<0.01$) in compared with control straw with 2% urea .Ammonia nitrogen concentration increased significantly ($p<0.01$) in straw with 2% urea compared with control after 48h. of incubation period while control treatment increased significantly ($p<0.01$) after 96h. incubation period compared with straw with 2% urea in ammonia nitrogen concentration, no significant differences found in ammonia nitrogen concentration after 24 and 72 h. incubation period between treatments, moreover, the in vitro dry matter digestibility, organic matter digestibility and metabilizable energy increased significantly ($p<0.01$) in barley straw with 2% urea compared with control. The result indicated that adding 2% urea to barley straw increased in vitro gas and methane production which lead to increased in vitro organic and dry matter digestibility and metabolizable energy.

**Keywards:** : gas production, methane production, barley straw, urea, in vitro digestibility.

**الخلاصة**

اجريت هذه التجربة لدراسة تأثير اضافة اليوريا (2%) الى تبن الشعير في بعض صفات التخمر (الاس الهيدروجيني وتركيز نتروجين الامونيا ) في المختبر ، انتاج الغاز الكلي وغاز الميثان في المختبر وقياس معامل الهضم المختبري للمادة الجافة والمادة العضوية والطاقة المتأيضة ، ثم اضافة اليوريا بنسبة 0 و 2% الى تبن الشعير المقطع مع المولاس بنسبة 4% وثم حضن العينات في حمام مائي بدرجة حرارة 39°م لمدة 24، 48، 72 و96 ساعة ، ودلت النتائج الى وجود انخفاض عالي المعنوية ($p<0.01$ ) في انتاج الغاز الكلي وغاز الميثان بعد فترات الحضن 48، 72 و96 لتبن الشعير مع اضافة 2% يوريا مقارنة مع عدم اضافة اليوريا الى التبن ، اما بعد الساعة 24من الحضن المختبري فقد ارتفع كل من الغاز الكلي وغاز الميثان بصورة عالية المعنوية ($p<0.01$ ) في التبن مع اضافة 2% يوريا مقارنة مع عدم اضافة اليوريا الى التبن، لوحظ من النتائج وجود ارتفاع عالي المعنوية ($p<0.01$ ) في الطاقة المتأيضة ومعامل الهضم المختبري للمادة العضوية والاحماض الدهنية قصيرة السلسلة والطاقة الصافية لإنتاج الحليب المقدرة من انتاج الغاز الكلي بعد 24 ساعة من الحضن المختبري في التبن المضاف له 2% يوريا مقارنة مع عدم اضافة اليوريا الى التبن ، واظهرت النتائج عدم وجود فروق معنوية ما بين المعاملات في قيمة الاس الهيدروجيني وفي فترات الحضن 24، 72 و96 ساعة ، اما فترة الحضن 48 ساعة فقد سجل اضافة 2% يوريا ارتفاع عالـــــــي المعنوية ($p<0.01$ )في قيمة الاس الهيدروجيني في حين اظهرت النتائج وجود ارتفاع عالـــــي المعنوية ($p<0.01$ ) في تركيز نتروجين الامونيا عند اضافة 2% يوريا الى التبن مقارنة مع معاملة السيطرة بعد فترة 48 ساعة، وتفوقت معاملة السيطرة ( $p<0.01$) بعد الساعة 96 مقارنة مع اضافة 2% يوريا الى تبن الشعير في تركيز نتروجين الامونيا بينما لم يحصل فرق معنوي بعد فترة الحضن 24 و 72 ، واشارت النتائج الى وجود ارتفاع عالـــــي المعنوية ($p<0.01$ ) عند اضافة 2% يوريا الى تبن الشعير مقارنة بمعاملة السيطرة في معاملة الهضم المختبري للمادة الجافة والمادة العضوية (%) والطاقة المتأيضة (ميكا جول/كغم مادة جافة) .وتبين نتائج هذه التجربة ان اضافة اليوريا بنسبة 2% ادت الى تقليل الغاز الكلي وغاز الميثان في المختبر ، مما انعكس على ارتفاع قيمة معامل الهضم المختبري للمادة الجافة والمادة العضوية والطاقة المتأيضة .

## Introduction

Rumen of the animal produced different types of gases as a result of a process of fermentation of various organic substances. These gases includes $H_2$, $H_2s$, CH4, and $Co_2$. methane (CH4) gas considered as an active mechanism to reduce co2 and eliminate h2 gas that develop in the rumen. the gases produced in ruminants varied according to several factors that includes ; the type of animal , the breed , pH of the rumen , percentages acetic acid , propionic acid , structure of ration , quantity of concentrate diet given to the animal (1)(2) . The production of CH4 gas in ruminants similar to that produced from organic fertilizer (3)(4). the CH4 having more temperature about 23 time than that of CO2 (5)(6) . It forms about 17.37 % from total gas production in ruminants animal and it's also forms about 8.12 % of the losses forms total energy of the ration and 11.13 % of the digestible energy (7). the in vitro production of gas give an indication to the type of ruminal fermentation , which is one of the fast method of low cost for determination of nutritive value of the ration (8) . cattle one of the first degree of producing CH4 then followed in sheep and then in goats (2) . in order to keep the energy of the ration through reduction of formation CH4 gas via addition of different feed additive which includes nutritive and not nutritive materials such as the use of antibiotics and feed additives (9). That lead to increase of feed intake, milk production and growth, but there was same limitation for their use because of its negative effect or its toxicity at its addition to the animal diet that leads to its effect on animal health and ruminal microbes which are deposited on milk and meat and a resistant to the antibiotics. for this reason this study was conducted to use an alternative like plant extracts and plant oils from medical plants (9) or an addition of non-protein nitrogenous sources such as urea in order to reduce CH4 .

## Materials and Methods

The experiment was conducted on nutrition lab. belonged to the department of animal production, college of Agriculture, university of Baghdad, AL-Jaderia collection. in order to study the effect of addition of urea 2% and the molasses in a percent of 4 % to the barley straw on some of ruminal fermentation and production of total gases and CH4 gas in vitro (lab) and the factor of digestibility in vitro to the dry matter, organic matter and the metabolic energy.

Studied traits:

Measurement of total gas production and CH4 in vitro:

Measarments of total gases in vitro have been carried out by taking 4 replicates for each sample according to (9). 200 mg were weighed from experimental nutritive materials added to 20 ml from synthetic saliva and 10ml from filtered (seaved) ruminal fluid and put it 100 ml glassware syringe then adding co2 gas to every syringe and then closed after removal of air and closed with plastic closure in order to prevent oozing of the fluid during incubator then the syringes were incubated in a waters bath at 37 c for 24 , 48 , 72 and 96 hours, and make a blank for each incubated period ( 4 replicates) , the syringes were with drawing to measure the total production of gas and then added 4 ml from sodium Hydroxide in a concentration of 4 % to two sample only to measure production of methane gas according to the method of (10).Then calculation of metabolic energy ( ME ) in ( megatol / kg of dry matter ) , the digestible factor in vitro for the organic matter % ( IVOMC ) , chain fatty acids ( mili moll litter ) ( SCFA) , the net energy of milk production ( NEL) from total gas production after 24 hours of incubator with the use of the following equestions :

ME ( MJ/Kg DM =1.06+0.157Gv +0.084cp- 0.081A (ASH) according to the method of menke and steingass(11).

IVOMD (%) = 14.88 + 0.889 GV = 0.45 CP + 0.651 XA (Ash) SCFA (mmol / leter ) = 0.0239 GV - 0.061 according to the method of menke and steingass (11).

NEL ( MJ/ Kg DM ) = 0.069 xGv + 0.0038 xcp + 0.000173 xEE2 +0.54 according to the method of (12) .

When :

ME= metabolic energy A= Ash %

G V = total gas production (ml) SCFA : short chain fatty acids

C P = crude protein 1 %

C F = crude fiber %

| Tries studies | Barley straw | |
|---|---|---|
| | Straw + molasses4% T1 | Straw+urea2%+ molasses4% T2 |
| Dry matter | 92.23 | 90.81 |
| Ash | 10.51 | 8.76 |
| Organic matter | 81.72 | 82. 05 |
| crude protein | 4.72 | 10.47 |
| crude fiber | 37.22 | 35.47 |
| Nitrogen-free extract | 39.59 | 35.28 |
| Ether extract | 0.19 | 0.83 |

Measurements of some traits of fermentation after each period of incubation :

These includes the measurements of pH and Amonia nitrogen .

In vitro measurement of factor of digestibility of dry and organic matter :

The measurements have been done according to the method of tilley and terry(13) .

Chemical Analysis :

Analysis of straw samples for determination of dry matter , organic matter , Ash , Ether extract , crude protein and crude fiber according to A.O.A.C (13) .

Statistical analysis :

The statistical analysis of the two experiments has been done according to the complete randomized design ( CRD ) in order to study the effect of treatment on different traits and made a comparison in signification difference between means using Duncan multiple range test (14). It is also used the SAS program for statistical analysis according to the following :

Yij = M + ti +oij

When :

Yij = value of studied trait

M = mean of studied trait

Ti = effect of treatment

Oij = random error when its distributed normally with a mean equal zero with a variance of Ge2

Table 1. Chemical composition of untreated barley straw with 4% molasses and 2% urea

## Results and Discussion

In vitro total gas and CH4 production (200mg dry matter /ml).

The result show (table-2) there was a significant decrease ($P < 0.01$) in total gas and CH4 production in barely straw with addition of 2% urea (T2) as compared with barely straw free from urea (T1) except at a period of 24 of in vitro incubation at which there was a decrease in total gas production and a volume of CH4 in straw not added to it the urea (T1) as compared with T2. it has been observed that was an increase in total gas production and CH4 progressively the time of incubation (24-96 hours) in vitro.

The addition of urea reduce the gas production ,but it increase with molasses addition when it is observed in the study on straw of sesame (15). When sesame straw treated with urea leads to decrease gas production as a result of increase protein percent (16) while with addition of molasses, similar results obtained by (17).

In vitro metabolizable energy and digestibility for organic matter , short chain fatty acid and net energy for milk production :

The result showed (Table-3) the presence of a high significant difference ($p < 0.01$) in metabolizable energy and digestibility in vitro for the organic matter, short chain fatty acids and the net energy for milk production of barely straw

added to it 2% urea (t2) as compared with the straw not added it urea (t1).

The of ME was 9.64 and 11.21 mega jol/kg of dry matter for treatments T1 and T2 respectively. while in vitro of digestibility factor for organic matter was 26.96 and 92.43 % for treatments T1 and T2 respectively and for short chain fatty acids 0.02 and 0.11 mli mol/ letter for T1 and T2 respectively. While the NE for milk production was 0.89 and 1.29 mega jol/ lg for dry matter respectively. the total gas and CH4 production after 24 hours in vitro incubation have indirect relationship with the metabolizable energy of the ration (18). Also the production of short chain fatty acids (12). This might be a good indicator for carbohydrate catabolism(19) that leads to measure the value of nutritive rations(20).the ME and DF in vitro for the organic matter increased after addition of the urea. Measurement of same fermentation traits after every incubation period :

The pH :

The result showed (Table-4) there was no significant difference in the value of pH between the two treatment (t1 , t2 ) in all in vitro incubation periods except at 48 hours when its showed high significant difference in barely straw with addition 2 % urea as compared with the control (T1) .

The addition of urea cause an increase in pH of rumen liquor but the addition of molasses reduce it. these result agreed with (15). When sesame straw treated with urea and molasses, From other hand addition of molasses causes production of lactic acid with a decreases in pH (21).

Ammonium nitrogen:

Table - 5 showed there was no significant difference an ammonia nitrogen concentration after 24, 72 hours of in vitro incubation between T1, T2 treatment while T2 showed a high significant difference ($p < 0.01$) in the conc. Of ammonia nitrogen (5.06 mg / 100 ml) as compared with T1 (barely straw without urea) (4.48mg /100 ml).

Table-5 also showed a significant decrease ($p <0.01$) in the conc. of ammonia nitrogen in barely with barely straw without addition of urea (5.02 and 5.7 mg/100 ml) respectively. The addition of urea to the barely straw cause an increase in the conc. of ammonia nitrogen. There was an increase in the conc. of ammonia nitrogen with increase in vitro incubation periods from 24.96 hours at T1 and T2 as a result of increase microbial digestion as incubation period increase. In vitro digestibility factor for dry an organic matter (%) and the metabolizable energy (mega jol/kg dry matter ):

The result showed (Table-6) a high significant difference ($p<0.01$) in vitro digestibility factor for dry and organic matter and the metabolizable energy for the barley straw added to it the urea with a means of 29.91, 26.29 %, 28.60, 25.14 % and 4.48, 4.03 mega jol /kg dry matter respectively. The addition of urea leads to an increase in the means of non-proteins nitrogen sources.

Table 2. Effect of urea supplementation (2%) and molasses (4%) on total gas and methane production (ml / 200 mg dry matter) in vitro for barley straw

| tries studies / Treatment types | Average ± standard error | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Total gas production | Methane production | Total gas production | Methane production | Total gas production | Methane production | Total gas production | Methane production |
| | The incubation period/ hour | | | | | | | |
| | 24 | 24 | 48 | 48 | 72 | 72 | 96 | 96 |
| T1 | 3.50±0.22b | 1.12±0.07b | 10.00±0.20a | 2.20±0.12a | 12.37±0.47a | 3.27±0.13a | 13.12±0.43a | 3.10±0.07a |
| T2 | 7.59±0.22a | 2.07±0.27a | 8.25±0.47b | 2.07±0.31ab | 8.94±0.21b | 2.57±0.43b | 10.37±0.55b | 2.97±0.31ab |
| Level of significant | ** | ** | ** | ** | ** | ** | ** | ** |

- T1 Straw + molass 4%, - T2 Straw + molass 4% + urea 2%.
**means there are high significant differences at the level of 0.01.

Table 3. Effect of urea addition (2%) on metabolic energy (MJ / kg dry matter), laboratory digestibility factor for organic matter (%), short chain fatty acids (mmol / L) and net energy for milk production (MJ / kg dry matter) Barley straw with molasses (4%) after 24 hours of laboratory incubation.

| tries studies / Treatment types | Average ± standard error | | | |
|---|---|---|---|---|
| | Metabolized energy (M j / kg dry material) | Laboratory digestibility factor for organic matter (%) | Short chain fatty acids (Mmol / L) | Net energy for milk production Mega joule / kg ) (dry material |
| T1 | 9.64±0.09 b | 26.96±0.20 b | 0.02±0.005 b | 0.89±0.02 b |
| T2 | 11.21±0.03a | 29.43±0.19 a | 0.11±0.005 a | 1.29±0.02 a |
| Level of significant | ** | ** | ** | ** |

- T1 Straw + molass 4%, - T2 Straw + molass 4% + urea 2%.
**means there are high significant differences at the level of 0.01.

Table 4. Effect of urea supplementation (2%) on barley straw pH with molasses addition (4%) after different laboratory incubation hours

| pH | | | | tries studies / Treatment types |
|---|---|---|---|---|
| Incubation period / hour | | | | |
| 96 | 72 | 48 | 24 | |
| 6.65 | 6.72 | 6.78b | 6.60 | T1 |
| 6.82 | 6.70 | 7.20a | 6.80 | T2 |
| NS | NS | ** | NS | Level of significant |

- T1 Straw + molass 4%, - T2 Straw + molass 4% + urea 2%.
**means there are a high significant differences at the level of 0.01, NS no significant differences.

Table 5. Effect of urea supplementation (2%) on barley straw in ammonia nitrogen concentration (mg / 100 ml) with molasses (4%) after different laboratory incubation hours

| tries studies / Treatment types | Average ± standard error | | | |
|---|---|---|---|---|
| | Ammonia nitrogen mg / 100 ml | | | |
| | Incubation period / hour | | | |
| | 24 | 48 | 72 | 96 |
| T1 | 3.83±0.22 | 4.48±0.20 b | 4.67±0.12 | 5.71±0.10 a |
| T2 | 4.08±0.06 | 5.06±0.37 a | 4.82±0.26 | 5.02±0.06 b |
| Level of significant | NS | ** | NS | ** |

- T1 Straw + molass 4%, - T2 Straw + molass 4% + urea 2%.
**means there are high significant differences at the level of 0.01, NS no significant differences.

Table 6. Effect of urea supplementation (2%) on barley adoption on laboratory digestion coefficient of dry matter, organic matter (%) and metabolic energy (MJ / kg dry matter) with molasses addition (4%).

| tries studies / Treatment types | Average ± standard error | | |
|---|---|---|---|
| | Laboratory digestibility coefficient of dry matter% | Laboratory digestibility factor for organic matter % | Metabolized energy (M joule / kg dry material |
| T1 | 26.89±0.40 b | 25.14±0.06 b | 4.03±0.06 b |
| T2 | 29.91±0.03 a | 28.60±0.05 a | 4.48±0.002 a |
| Level of significant | ** | ** | ** |

- T1 Straw + molass 4%, - T2 Straw + molass 4% + urea 2%.
**means there are high significant differences at the level of 0.01.
Metabolic energy = 0.15 × laboratory digestion coefficient of organic matter(22).

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