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IN *VITRO* GAS PRODUCTION OF SOME ROUGHAGES USING DIFFERENT LEVELS OF AWASSI LAMBS AND BUFFALOES RUMEN FLUID.

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ABSTRACT

This study was conducted at the College of Agricultural Engineering Sciences/university of Baghdad. Were used wild reeds and barley straw inoculation with cumulative levels of rumen fluid, Which aimed to calculate in *vitro* total and methane gas production using five treatments, as follows-:

T1: 1 ml of buffalo rumen fluid + 9 ml of lamb rumen fluid.
T2: 2 ml of buffalo rumen fluid + 8 ml of lamb rumen fluid.
T3: 4 ml of buffalo rumen fluid + 6 ml of lamb rumen fluid.
T4: 6 ml of buffalo rumen fluid + 4 ml of lamb rumen fluid.
T5: 8 ml of buffalo rumen fluid + 2 ml of lamb rumen fluid.

The results showed a highly significant decreases (P≤0.01) in total gas and methane production for the treatments T1, T2, and T3 after 24, 48, and 72 hr. of incubation periods compared with, T4 and T5 for wild reed and barley straw samples. The results also showed a highly significant decreases (P≤0.01) in the production of total gas and methane production after, 48 hr. of incubation periods of barley straw samples compared with wild reeds. The results also showed a highly significant increases (P≤0.01) in the values of the organic matter digestibility, metabolizable energy, and net energy for milk production for treatments T4 and T5 compared to treatments T1, T2, and T3 after 24 hr. of incubation period. Conclude be that addition buffalo rumen fluid didn't decrease of total gas and methane production in in *vitro*, so this means that buffalo are less efficient than sheep in utilizing feed energy.

Key words: methane gas, wild reeds, metabolizable energy, net energy for milk production.

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INTRODUCTION

Sheep have an important place in Iraq among farm animals and are considered one of the most important in terms of their contribution to income, it is necessary to pay attention to sheep and increase their productivity by improving rumen fermentations, which is reflected positively in reducing the energy lost in the form of gases, as good rumen fermentations can serve as promising strategies for reducing greenhouse gas emissions. Several technologies have been used in this field to improve rumen fermentations and feed digestibility. Treatment with oyster mushrooms improved the fermentation efficiency of silage made from barley straw (Hassan & Al-Samaraee, 2015, 2020), and treatment with Trichoderma harzianum also improved the in *vitro* digestibility of dry matter (DM) and organic matter (OM) of roughage (Al-Samaraee et al., 2019), and treatment with probiotics and bread yeast improved rumen fermentations and the nutritional value of barley straw (Alwaeli et al., 2017; Al-Samaraee et al., 2019). The concentration of ammonia in the rumen decreased significantly when protected lysine was added to the ration of local ewes with a

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significant improvement in rumen fermentations, which may be reflected positively in reducing methane production. Reducing methane emissions from ruminants is an important goal to reduce the environmental impact of agriculture, as studying greenhouse gas emissions is of great importance, increasing their concentrations in the environment leads to serious risks summed up in climate change, as increasing their emissions is still a source of concern due to their negative effects on the phenomenon of global warming and the rise in climate change and temperatures (Caro et al., 2014). Methane contributes 4-9% of the total gases (Moss et al., 1995) and approximately 15% of greenhouse gas emissions are generated by livestock (Króliczewska et al., 2023) as a by-product of the microbial digestion of feed, and ruminants are among the largest sources of methane production. (Chang et al., 2021). Sheep contribute less to gas emissions than large ruminants. Several strategies can be used to reduce greenhouse gas emissions, such as dietary modifications, genetic selection, and inoculation of rumen microbiota, use of plant secondary metabolites, methane inhibitors, and algae (Króliczewska et al., 2023). Food additives have been used, such as vegetable oils to reduce methane production (Hassan & Irhaeem, 2016), castor and flax oil (Hassan & Abd Alah, 2018; Kuttar et al., 2017, 2018), and garlic powder at different levels (Mohammad & Alsahaf, 2022), which significantly affected the total gas produced and the reduction of methane gas. As methane production decreased in the in *vitro* when treating the feed with Al-Bassr date powder (Hassan & Abd Alah, 2020). Non-nutritive feed additives such as nitrate and urea (Hussein & Hassan, 2019) and flavonoids extract from mulberry leaves (AL-Bayati & Hassan, 2018) were also used, and the feed was treated with a mixture of Albizia Falcataria with condensed tannin content, as well as adding different levels of nano-zinc oxide to date by-products (Kadhim Al-ghazali & Muhammad, 2022). These treatments and methods may be considered promising technologies for reducing total gas and methane gas production. Given the lack of studies on the effect of inoculation with rumen fluid in reducing greenhouse gas emissions, we are here in the process of studying the effect of inoculation with lamb rumen fluid on the in *vitro* of methane and total gases in their counterparts in buffalo.

MATERIALS AND METHODS

This study was conducted at the College of Agricultural Engineering Sciences / University of Baghdad for the period from 17 to 24/4/2022 to know the effect of inoculating the rumen fluid of Awassi lambs with its counterpart in buffalo on in *vitro* total gases and methane production. Awassi lambs and Iraqi buffalo rumen fluid were collected directly after slaughter from adult animals. In this study, 5 treatments (T1, T2, T3, T4, and T5) with 3 replicates for each treatment, representing 5 inoculation ratios, by forming a mixture of the two liquids for each treatment were used according to the following ratios**:**

T1:1 ml of buffalo rumen fluid + 9 ml of lamb rumen fluid**.**

T2: 2 ml of buffalo rumen fluid + 8 ml of lamb rumen fluid

T3: 4 ml of buffalo rumen fluid + 6 ml of lamb rumen fluid

T4: 6 ml of buffalo rumen fluid + 4 ml of lamb rumen fluid

T5: 8 ml of buffalo rumen fluid + 2 ml of lamb rumen fluid **.**

These percentages of fluid were added to 100 ml graded glass syringes containing 200 mg of dried and crushed samples of (Iraqi wild reeds and barley straw) to measure the in *vitro* of total gases and methane production, to study the effect of inoculating the rumen fluid of Awassi lambs with its counterpart in buffalo and effect of Iraqi wild reeds and barley straw according to the following steps below**:**

Steps of estimation of total gas and methane production**:** The process of estimating methane production and total gases was carried out in the in *vitro* by taking 3 replicates of 200 mg dried and crushed samples each from Iraqi wild reeds and barley straw, and placing them in glass syringes for each of the five treatments mentioned above, according to the method of Menke (1988), and 20 ml of artificial saliva prepared in *vitro* and 10 ml of the mixture prepared from the two rumen liquids (sheep rumen liquid + buffalo rumen liquid) according to the proportions calculated for each of the five treatments, filtered and taken from the rumen of adult sheep and Iraqi buffaloes immediately after slaughter, Then carbon dioxide gas was added only once to each injection immediately before incubation. The syringes were closed with the plunger and pushed up to get rid of the syringe needle was completely sealed with a rubber stopper to prevent liquid from leaking during incubation. It was incubated in a water bath at a temperature of 39°C for 24, 48, and 72 hours, with a plank being made for each stage of incubation (5 replicates), and the syringes were withdrawn to calculate gas production. Then, 4 ml of 4% sodium hydroxide was added to the injection to calculate methane production according to the method of Fievez et al **.**(2005) **.**The coefficient of digestion of organic matter in the laboratory, the metabolic energy and the net energy for milk production were also calculated according to the following equations**:**

ME (MJ/kg DM) = 1.06 + 0.157GV + 0.084CP + 0.22CF −0.081A (Ash). (Menke, 1988)**.**

IVOMD (%) = 14.88 +0.889 GV + 0.45CP + 0.651 x A (ASH). (Menke, 1988) **.**

NEL (MJ/Kg DM) = 0.096xGV + 0.0038xCP + 0.000173x EE2+0.54

Since**-:**

A = ash %. GV = total gas production (ml)**.**

CP = crude protein %. CF = Crude Fiber**.%**

EE = ether extract**.%**

Statistical analysis: The statistical analysis was conducted according to the Completely Randomized Design (CRD) to study the effect of the treatment on the different traits, and the significant differences between the means were compared with the Duncan's multiple range test (Duncan, 1955), multinomial test, and the ready-made program SAS (2012), was used in the statistical analysis.

RESULTS AND DISCUSSION

In *vitro* gas and methane production of Iraqi wild reeds inoculating in Awassi lambs and buffalo rumen fluid: The results in Table 1 showed that total gas production after 24 hr. of in *vitro* incubation period, significantly decreased ($P \leq 0.01$) were 14.53, 15.40, and 16.53 ml for T1, T2, and T3 respectively, compared with T4 and T5, were 21.86 and 20.66 ml, respectively. While Methane production decreased significantly ($P \leq 0.01$) in T1, T2, and T3 after 24 hr. of incubation period (3.72, 3.94, and 4.23 ml respectively) compared with T4 and T5 (5.59 and 5.29 ml). The results in Table (1) also show that after 48 hr. of incubation period total gas production in T1, T2, and T3, decreased significantly ($P \leq 0.01$) to give the value 18.93, 20.00, and 21.06 ml compared with T4 and t5 (27.26 and 25.46 ml), while T1, T2, and T3 recorded significant ($P \leq 0.01$) decrease in methane production (6.89, 7.16, and 7.44 ml respectively) compared with T4 and T5 (9.02 and 8.56 ml respectively). Treatments T1, T2, and T3 result decreased significantly ($P \leq 0.01$) in total gas production (20.03, 20.83, and 21.62 compared with 26.23 and 24.89 ml for T4 and T5, while methane production decrease significantly ($P \leq 0.01$) in T1, T2, and T3 (5.12, 5.33, and 5.53 ml), compared with T4 and T5 (6.71. and 6.37 ml) respectively, after 72 hr. of incubation period. The reason for the increase in the total gas and methane production is due to the interaction between the effect of inoculation and time (McDermott et al., 2020). These results are consistent with Belanche et al. (2015) which showed that inoculating the rumen with protozoa and microorganisms has a significant impact on increasing methane concentrations. The results were consistent with Hassan (2014) which showed that the increase in total gases production and methane gas in particular increases with increasing duration in vitro incubation. The increase may be due to the improvement of digestion and fermentation and the availability of sufficient time for the effectiveness and activity of rumen microorganisms, which may lead to an increase in the production of gases as a by-product of the digestion process. Martin et al., (2010) demonstrated that variation among animals in methane production within the same level of performance and using similar diets was high. Morgavi et al. (2010) Showed that the decrease in methane emissions should be attributed to changes in rumen microbes and the availability of H2, as he explained that

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there is a direct link between the number of protozoa and methane production, showing that it is possible to modify the rumen microbial ecosystem to reduce its production of methane.

Table 1. In *vitro* gas and methane production of Iraqi wild reeds (ml/200 mg DM) inoculating in Awassi lambs and buffalo rumen fluid (Means ± standard error).

Traits	Total gas volume (ml)	Methane gas volume (ml)	Total gas volume (ml)	Methane gas volume (ml)	Total gas volume (ml)	Methane gas volume (ml)
Treatments	Means ± standard error and Incubation period					
	24	24	48	48	72	72
T1	14.53 ± 0.48 c	3.72 ± 0.12 c	18.93 ± 0.48 b	6.89 ± 0.12 b	20.03 ± 0.35 b	5.12 ± 0.09 b
T2	15.40 ± 0.72 cb	3.94 ± 0.18 cb	20.00 ± 0.83 b	7.16 ± 0.21 b	20.83 ± 0.61 b	5.33 ± 0.15 b
T3	16.53 ± 0.96 b	4.23 ± 0.24 b	21.06 ± 1.09 b	7.44 ± 0.27 b	21.62 ± 0.81 b	5.53 ± 0.20 b
T4	21.86 ± 0.13 a	5.59 ± 0.03 a	27.26 ± 0.76 a	9.02 ± 0.19 a	26.23 ± 0.57 a	6.71 ± 0.14 a
T5	20.66 ± 0.35 a	5.29 ± 0.09 a	25.46 ± 0.35 a	8.56 ± 0.09 a	24.89 ± 0.26 a	6.37 ± 0.06 a
Significantly level	**	**	**	**	**	**

The averages with different letters within the same column are significantly different for each factor. ** (P≤0.01).

***(T1, T2, T3, T4, and T5) what means below the table: T1: 1 ml of buffalo rumen fluid + 9 ml of lamb rumen fluid**
T2: 2 ml of buffalo rumen fluid + 8 ml of lamb rumen fluid., T3: 4 ml of buffalo rumen fluid + 6 ml of lamb rumen fluid
T4: 6 ml of buffalo rumen fluid + 4 ml of lamb rumen fluid., T5: 8 ml of buffalo rumen fluid + 2 ml of lamb rumen fluid

In *vitro* gas and methane production of Barley Straw inoculating in Awassi lambs and buffalo rumen fluid: The results in Table (2) show that total gas production after 24 hr. of in *vitro* incubation period, significantly decreased (P≤0.01) were 11.03, 13.68 ml for T1 and T2, respectively, compared with T3, T4, and T5, were 17.80, 18.88 and 19.85 ml, respectively. While Methane production decreased significantly (P≤0.01) in T1 and T2, after 24 hr. of the incubation period (2.82 and 3.50ml) respectively, compared with T3, T4, and T5, were 4.55, 4.83, and 5.08 ml. The results of inoculation with buffalo rumen fluid, as the treatments T1, T2, and T3 contributed to reducing the production of total gas and methane significantly (P≤0.01) after 48 and 72 hours of incubation periods, reached 14.49 and 18.15 ml for the total gas and 3.711, and 4.64 for methane for (T1). While the total gas production for (T2) reached 15.45 and 19. 45 ml respectively. As for methane gas production, it reached 3.95, and 4.98 ml, respectively. As for the third treatment (T3), the total gas production reached 16.57, and 20.57 ml, while the methane gas production reached 4.24, and 5.26 ml for the periods 48, and 72 hours of incubation, respectively. At the same time, the production of total and methane gas as a result of inoculation with buffalo rumen fluid increased significantly (P≤0.01) for treatments, T4 and T5 with the volume were 18.88, 22.38, and 26.38 ml for total gas compared to the other treatments, with volume 4.83, 5.72. And 6.75 ml for methane gas after 24, 48 and 72 hours of incubation periods respectively, for (T4). Also total gas were 19.84, 20.68, and 24.68 ml, while the methane gas volume were 5.08, 5.79, and 6.91 ml after 24, 48, and 72 hours of incubation periods respectively for (T5). The reason for the increase in the production of total and methane gas after 48 and 72 hours of the incubation period is due to the inoculation with buffalo rumen fluid, which enhanced from the microbial digestion of Iraqi wild reeds and barley straw, which was reflected in the in *vitro* production of total and methane gas (Keady et al., 2012; Al-Nadawi & Al-Samarrai, 2024a, 2024b) showing that use of bacterial inoculants is expected to lead to an improvement in the performance of animal.

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While some strains of lactic acid bacteria (*Lactobacillus plantarum*) have been shown to reduce methane production (Cao et al., 2011). In another study from the same group, Cao et al. (2010) reported similar results with the same inoculation strain with vegetable residue silage. Methane gas is affected by the digestibility coefficient of nutrients, which is affected by the time of harvest and the degree of maturity (Gerber et al., 2013; Kim et al., 2011), and methane production can be reduced by grinding feed (Johnson & Johnson, 1995), due to the reduced residence time of the feed in the rumen compared to chopped roughages feeds (Moss et al., 1995). Several factors cause fluctuation in methane production, such as the amount of intake, type of fermentable carbohydrates, feed manufacturing, and the addition of lipids (Johnson & Johnson, 1995). Roughages feed also leads to higher methane production compared to Concentrated (Hindrichsen et al., 2006), as replacing concentrated feed with straw led to increased methane production (Külling et al., 2002). Increased DMI is also associated with increased methane production (Hegarty et al., 2010). In the current study, the treatments T1, T2, and T3 were the best in terms of their contribution to reducing gas production.

Table 2. In *vitro* gas and methane production of barley straw (ml/200 mg DM) inoculating in Awassi lambs and buffalo rumen fluid (Means ± standard error).

Traits	Total gas volume (ml)	Methane gas volume(ml)	Total gas volume (ml)	Methane gas volume(ml)	Total gas volume (ml)	Methane gas volume(ml)
Treatments	Means ± standard error and Incubation period					
	24	24	48	48	72	72
T1	11.03 ± 1.10 b	2.82 ± 0.28 b	14.49 ± 0.48 b	3.71 ± 0.12 b	18.15 ± 0.49 c	4.64 ± 0.12 c
T2	13.68 ± 0.62 b	3.50 ± 0.16 b	15.45 ± 0.73b	3.95 ± 0.18 b	19.45 ± 0.73 bc	4.98 ± 0.18 bc
T3	17.80 ± 0.42 a	4.55 ± 0.10 a	16.57 ± 0.96 b	4.24 ± 0.24 b	20.57 ± 0.96 b	5.26 ± 0.24 b
T4	18.88 ± 0.98 a	4.83 ± 0.25 a	22.38 ± 0.74 a	5.72 ± 0.18 a	26.38 ± 0.74 a	6.75 ± 0.18 a
T5	19.84 ± 1.17 a	5.08 ± 0.29 a	20.68 ± 0.32 a	5.79 ± 0.09 a	24.68 ± 0.32 a	6.91 ± 0.09 a
Significantly level	**	**	**	**	**	**

The averages with different letters within the same column are significantly different for each factor. ** (P≤0.01).

In *vitro* gas and methane production of Iraqi wild reeds and barley straw inoculating in Awassi lambs and buffalo rumen fluid The results in Table (3) show a highly significant increase (P≤0.01) in the production of total and methane gas after 48 hours of incubation period of Iraqi wild with, 22.54 and 7.81 ml respectively, compared to barley straw, with 17.91 and 4.68 ml respectively. The results in Table 3 also show that there were no significant differences in total gases and methane production due to the effect of feed type after 24 and 72 hours of incubation periods. These results are consistent with what was reported by Króliczewska et al. (2023) who showed that feeding cellulose leads to increased intestinal methane emissions with great variation due to the type and source of the feed, chemical composition, and digestibility. The results are also consistent with Alemu et al. (2011) and Shibata and Terada (2010), who stated that feed quality affects methane production in ruminants, and different feed types can also affect methane emissions due to differences in their chemical composition (Benchaar et al., 2001), as eating roughages feed leads to higher methane production compared to concentrated feed (Hindrichsen et al., 2006), and replacing concentrated feed with straw led to an increase in methane production (Külling et al., 2002). High-quality forages, such as young plants, can reduce methane production by altering the fermentation pathway because they contain greater amounts of easily fermentable carbohydrates and less NDF, leading to increased digestibility and passage rate through the rumen. In contrast, more mature

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forages result in higher methane production mainly due to an increased C: N ratio which reduces digestibility (Milich, 1999), while younger forages have lower fiber content and higher soluble carbohydrate content. Supplementing them with a small amount of grains is a promising mitigation approach to reduce greenhouse gas emissions (Haque, 2018). From the above, inoculation with rumen fluid or microbial enhancers can reduce methane production and can be used as a safe biological means to reduce methane emissions and manipulate the microbial community, when cross- inoculation the rumen of buffalo with its counterpart in sheep. Inoculation the rumen ecosystem with probiotics also supports rumen fermentations and increases feed efficiency, which may reduce methane emissions (Islam & Lee, 2019), as there is a well-established relationship between increased propionate production and reduced methane emissions, (Haque, 2018). The reason for the increase in methane gas production may be due to the indirect effect of hydrogen gas production as a result of increased fiber digestion, which affects the number and efficiency of rumen microflora.

Table .3. In *vitro* gas and methane production wild reeds and barley straw (ml /200 mg DM) inoculating in Awassi lambs and buffalo rumen fluid (Means ± standard error).

Traits	Total gas volume (ml)	Methane gas volume(ml)	Total gas volume (ml)	Methane gas volume(ml)	Total gas volume (ml)	Methane gas volume(ml)
Treatments	Means ± standard error and Incubation period					
	24	24	48	48	72	72
wild reeds	17.8 ± 0.81	4.55 ± 0.20	22.54 ± 0.91 a	7.81 ± 0.23 a	22.72 ± 0.67	5.81 ± 0.17
Barley straw	16.25 ± 0.95	4.16 ± 0.24	17.91 ± 0.86 b	4.68 ± 0.24 b	21.84 ± 0.88	5.71 ± 0.25
Significantly level	NS	NS	**	**	NS	NS

The averages with different letters within the same column are significantly different for each factor. ** (P≤0.01), NS: not significant.

In *vitro* (IVOMD) %, Metabolizable energy (MJ/kg DM) and Net energy of milk production NEL (MJ/Kg DM) of Iraqi wild reeds inoculating in Awassi lambs and buffalo rumen fluid: The results in Table (4) show the presence of highly significant differences (P≤0.01), as the values of the in *vitro* organic matter (IVOMD) % digestibility coefficient, metabolizable energy (MJ/kg DM), and net energy for milk production (NEL) for Iraqi wild reeds after 24 hr. of incubation period was highly significant (P≤0.01), for T4 and T5 in IVOMD %, were 42.73 and 41.66%, while the metabolizable energy values were 11.30 and 11.11MJ/kg DM, and the net energy values for milk production were 2.66 and 2.54 MJ/kg DM respectively, compared to the treatments T1, T2 and T3, which decreased significantly (p<0.01)) with values reaching 36.21, 36.98 and 37.99% for the IVOMD %. While the values of metabolizable energy were 10.15, 10.28, and 10.46 MJ/kg DM. The net energy values for milk production were 1.95, 2.04, and 2.14 MJ/kg DM for T1, T2, and T3, respectively. The net energy values for milk production were fairly similar. It is noted from the values in Table 4, that the effect of in *vitro* incubation with a mixture of rumen fluid from Awassi lambs and buffaloes had a clear effect in increasing the values of the IVOMD %, metabolizable energy, and net energy for milk production. Also, these values are usually affected by the production of total gas and methane gas after 24 hours of incubation period, as it has an indirect relationship with the energy metabolized in the feed (Menke et al., 1979), although methane production after 24 hours of incubation period, does not correspond to the rise and fall of feed metabolizable energy values for calculations based on the total gas volume produced. It may be the reason for increasing metabolizable energy values may be a result of the significant (P≤0.01) improvement IVOMD %.

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Table 4. In *vitro* (IVOMD) %, Metabolizable energy) MJ/kg DM) and Net energy of milk production NEL (MJ/Kg DM) of Iraqi wild reeds (ml/200 mg DM) inoculating in Awassi lambs and buffalo rumen fluid (Means ± standard error).

Factors / Adjectives	Means ± standard error		
	(IVOMD)%	Metabolizable energy (ME) (MJ/kg DM)	Net energy of milk production NEL (MJ/Kg DM)
T1	36.21±0.42c	10.15±0.07c	1.95±0.04c
T2	36.98±0.64cb	10.28±0.893cb	2.04±0.06cb
T3	37.99 ± 0.85b	10.46 ± 0.15b	2.14 ± 0.09b
T4	42.73 ± 0.11 a	11.30 ± 0.02 a	2.66 ± 0.01 a
T5	41.66 ± 0.31 a	11.11 ± 0.05 a	2.54 ± 0.03 a
significant level	**	**	**

The averages with different letters within the same column are significantly different for each factor. ** (P≤0.01), NS: not significant.

In *vitro* (IVOMD)%, Metabolizable energy (MJ/kg DM)and Net energy of milk production NEL (MJ/Kg DM) of barley straw inoculating in Awassi lambs and buffalo rumen fluid: The results in Table (5) show the presence of highly significant differences (P≤0.01), as the values of IVOMD %, metabolizable energy MJ/kg DM, and net energy for milk production (NEL) for Barley straw after 24 hr. of incubation period, with highly significant (p<0.01)) for the treatments T3, T4, and T5 that were 38.49, 39.45 and 40.31%, while the metabolizable energy values reached 11.84, 12.01 and 12.16 MJ/kg DM. As for the net energy values for milk production, they were 2.26, 2.36, and 2.46 MJ/kg DM, respectively, compared to T1 and T2, which decreased significantly (P≤0.01) with values reaching 32.47 and 34.83% for the *in vitro* digestibility coefficient, while the values of the metabolizable energy values were 10.78 and 11.19 MJ/kg DM, while the net energy values for milk production were 1.61 and 1.86 MJ/kg DM, respectively. The net energy values for milk production were fairly similar. It is noted from the values in Table 5 that the effect of in *vitro* incubation with a mixture of rumen fluid from Awassi lambs and buffaloes had a clear effect in increasing the values of the IVOMD %, metabolizable energy, and net energy for milk production of barley straw. Also, these values are usually affected by the production of total gas and methane gas after 24 hr. of in *vitro* incubation, as it has an indirect relationship with the energy metabolized in the feed (Menke et al., 1979), although methane production after 24 hr. of in *vitro* incubation does not correspond to the rise and fall of Feed metabolizable energy values for calculations based on the total gas volume produced. The reason for the increase in metabolizable energy values may be a result of the improvement in the IVOMD %.

Table 5. In *vitro* (IVOMD) %, Metabolizable energy) MJ/kg DM) and Net energy of milk production NEL (MJ/Kg DM) of barley straw (ml/200 mg DM) inoculating in Awassi lambs and buffalo rumen fluid (Means ± standard error).

Factors / Adjectives	Means ± standard error		
	(IVOMD)%	Metabolizable energy (ME) (MJ/kg DM)	Net energy of milk production NEL (MJ/Kg DM)
T1	32.47 ± 0.17b	10.78 ± 0.17b	1.61 ± 0.10b
T2	34.83 ± 0.55 b	11.19 ± 0.09 b	1.86 ± 0.06 b
T3	38.49 ± 0.37a	11.84 ± 0.06a	2.26 ± 0.04a
T4	39.45 ± 0.87 a	12.01 ± 0.15 a	2.36 ± 0.09 a
T5	40.31 ± 1.04a	12.16 ± 0.18a	2.46 ±0.11a
significant level	**	**	**

The averages with different letters within the same column are significantly different for each factor. ** (P≤0.01), NS: not significant.

CONCLUSION

The study concludes that increasing the proportion of buffalo rumen fluid (T4 and T5) significantly increases total gas and methane production compared to sheep-dominant treatments. While these treatments showed

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higher organic matter digestibility and energy values, the elevated gas output indicates a lower efficiency in energy utilization. Consequently, buffalo appear less efficient than sheep at converting feed energy, as the addition of their rumen fluid failed to mitigate methane emissions during the fermentation of wild reeds and barley straw.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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إنتاج الغاز المختبري لبعض الأعلاف الخشنة بإستخدام مستويات مختلفة من سائل كرش الحملان العواسية والجاموس.

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المستخلص

أُجريت الدراسة في كلية علوم الهندسة الزراعية / جامعة بغداد. أُستخدم فيها عينات من القصب البري وتبن الشعير مع نسبٍ تصاعدية من سائل الكرش ، والتي هدفت إلى حساب إنتاج الغاز الكلي وغاز الميثان مختبرياً للمعاملات الآتية:

T1: 1 مل من سائل كرش الجاموس + 9 مل من سائل كرش الحملان.

T2: 2 مل من سائل كرش الجاموس + 8 مل من سائل كرش الحملان.

T3: 4 مل من سائل كرش الجاموس + 6 مل من سائل كرش الحملان.

T4 : 6 مل من سائل كرش الجاموس + 4 مل من سائل كرش الحملان.

T5: 8 مل من سائل كرش الجاموس + 2 مل من سائل كرش الحملان.

وقد أظهرت النتائج وجود إنخفاض عالي المعنوية (P≤0.01) في إنتاج الغاز الكلي وغاز الميثان للمعاملاتT1 ، T2 وT3 بعد 24 ، 48 و72 ساعة من الحضن المختبري مقارنة بالمعاملتين T4 وT5 لعينات القصب البري وتبن الشعير. وأظهرت النتائج أيضاً إنخفاضاً عالي المعنوية (P≤0.01) في إنتاج الغاز الكلي وغاز الميثان بعد (48) من الحضن المختبري لعينات تبن الشعير مقارنة بالقصب البري. كما أظهرت النتائج إرتفاعاً عالي المعنوية (P≤0.01) في قيم معامل هضم المادة العضوية ، الطاقة المتأيضة والطاقة الصافية لإنتاج الحليب للمعاملتين T4 وT5 مقارنة بالمعاملات T1 ، T2 وT3 بعد 24 سلعة من الحضن المختبري. نستنتج من ذلك بأن إضافة سائل كرش الجاموس لم تؤثر في تقليل إنتاج الغاز الكلي وغاز الميثان مختبرياً ، وهذا يعني أن الجاموس أقل كفاءة من الأغنام في الإستفادة من طاقة العلف.

الكلمات المفتاحية: المجترات، غاز الميثان، القصب البري، الطاقة المتأيضة، الطاقة الصافية لإنتاج الحليب.