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Effects of mealworm (Tenebrio Molitor L.) larvae supplementation to alfalfa hay at different levels on in vitro gas and methane production

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Keywords

Edible insects, Ruminant, Feed, Gas production, Methane

Abstract

The study was conducted to determine the effects of adding different levels of mealworm (Tenebrio Molitor L.) larvae to alfalfa hay on in vitro gas and methane production. For this purpose, mealworms were added to alfalfa hay at the rate of 0.5%, 1%, and 1.5% of the dry matter. In the study, rumen fluid was obtained from the slaughterhouse (2 healthy beef cattle). In the study, gas production was found to be higher in the groups supplemented with mealworms (PL<0.05, PQ<0.05). Similarly, in vitro, organic matter digestibility and net energy for lactation increased in groups supplemented with mealworm larvae compared to the control (PL<0.05, PQ<0.05). While metabolic energy values increased linearly with the addition of mealworms, the quadratic effect was found to be insignificant. The effect of mealworm addition to alfalfa hay on in vitro methane production was found to be insignificant (P>0.05). As a result, it was determined that the use of mealworm larvae as a feed additive in ruminant feeds could increase the digestibility of alfalfa without affecting the methane production. It is also predicted that mealworms may increase the sustainability of ruminant feeding. However, it is recommended that these results be supported by in vivo studies at different levels.

Anahtar Kelimeler

Yenilebilir böcek, Ruminant, Yem, Gaz üretimi, Metan

Yonca kuru otuna farklı oranlarda un kurdu (Tenebrio Molitor L.) larvası ilavesinin in vitro gaz ve metan üretimine etkisi

Özet

Bu çalışma yonca kuru otuna farklı oranlarda un kurdu (Tenebrio Molitor L.) larvası ilavesinin in vitro gaz ve metan üretimine etkilerini belirlemek amacıyla yapılmıştır. Bu amaçla yonca kuru otuna kuru maddede %0.5, %1 ve %1.5 oranında un kurdu ilave edilmiştir. Çalışmada rumen sıvısı kesimhaneden (2 adet sağlıklı besi sığırından) elde edilmiştir. Çalışmada gaz üretimi un kurdu ilave edilen gruplarda daha yüksek bulunmuştur (PL<0.05, PQ<0.05). Benzer şekilde in vitro organik madde sindirilebilirliği ve laktasyon için net enerjisi un kurdu larvası ilave edilen gruplarda kontrole kıyasla artmıştır (PL<0.05, PQ<0.05). Metabolik enerji değerleri ise lineer olarak un kurdu ilavesi ile artış gösterirken, kuadratik etkisi önemsiz bulunmuştur. Yonca kuru otuna un kurdu ilavesinin in vitro metan üretimine etkisi önemsiz bulunmuştur (P>0.05). Sonuç olarak un kurdu larvasının ruminant yemlerinde yem katkı maddesi olarak kullanımı ile yoncanın sindirilebilirliğinin metan üretim oranını etkilemeksizin artırabileceği belirlenmiştir. Ayrıca un kurdunun ruminant beslemenin sürdürülebilirliğini artırabileceği öngörülmektedir. Ancak bu sonuçların farklı dozlarda ve in vivo çalışmalar ile desteklenmesi önerilmektedir.

Bu makale Merve Hanönü isimli öğrencinin yüksek lisans tezinden üretilmiştir.

1. INTRODUCTION

It is estimated that animal derived products consumption will increase by 60-70% by 2050. Although provide the increasing consumption pushes the search for more resources, the sustainability of feed resources has become a difficult issue for many reasons such as the limited availability of these resources, the environmental effects of production, climate change, and the food-feed-fuel race. For this reason, searching for alternative sources instead of traditional feed sources has become more important. Studies on the use of edible insects as alternative feed have gained momentum in recent years. Although the use of insects in animal feeding is seen as promising, there are many parameters that need to be investigated.

In the last years, scientific studies regarding the use of insects both as feed and food has continued exponentially. The industrial sector is also increasingly interested in growing, processing and marketing edible insect species (van Huis 2019). These farmed insects have important advantages such as being able to be grown in small areas, being propagated in semi-waste or completely waste products that are not suitable for human consumption, and often meeting the water they need from the food they consume. Considering the areas allocated for the cultivation of forage crops and their water needs, it seems that the cultivation of insects is much more economical (Canhilal ve ark., 2020). Insects that have high feed utilization also grow very quickly. Approximately 2 kg of organic residue and 1 m2 of area may be sufficient to create one kg of insect protein (Odabaşı and Yeşilbağ, 2021). Other reasons why insects attract great attention as an alternative protein source include, they are sustainable, have good feed conversion levels, and have high levels of protein (30%-70% in dry matter) content, which varies on a wide scale (Işık and Kırkpınar, 2016). While protein-rich insects are seen as an alternative to reduce the cost of protein additives in rations, they are also very rich in fat, minerals, vitamins and essential amino acid contents. In fact, it is compared to fish meal and soybean meal in terms of protein quality.

Global warming is seen as a huge threat to the future of the world. Especially the high levels of methane gas (CH4) formed because of rumen fermentation will become a much more effective problem due to the increase in human population and animal numbers. In addition to global warming, methane causes the loss of 3% of the energy consumed in animals fed with concentrated feed and 12% of the energy consumed in animals fed with roughage (Gür and Öztürk, 2021). These negative effects of methane emissions have led the industry and academic circles to find new and effective alternative feed sources (Demirci and Yetim, 2021). For the last decade, insects have been considered as alternative feed ingredients for ruminants, and interest in their use for animal feed has increased due to their high nutritional properties and environmental advantages compared to other animal feeds (Ahmed ve ark., 2021). Especially the oils found in insects reduce enteric methane emissions when added to feed and highly concentrated diets (Jayanegara ve ark., 2020). Although insects have been used effectively in various animal diets, their evaluation in ruminant animals has been limited.

2. MATERIAL VE METHODS

Alfalfa hay and mealworm larvae were used as materials in the experiment. Mealworm larvae were purchased from a business operating in Ankara. Organic matter digestibility and methane production were determined by in vitro gas production method by adding different amounts (0.5, 1 and 2%) to alfalfa hay after it was dried and ground.

The technique reported by Menke ve ark., (1979) was used to determine the digestibility and energy due to in vitro gas production in the experiment. Accordingly, all samples were placed in special glass syringes (Model Fortuna, Häberle Labortechnik, Lonsee-Ettlenschie β, Germany) with a volume of 100 ml, weighing around 200±10 mg DM in three parallel ways. 30 ml of rumen fluid/buffer solution (Menke and Steingass, 1988) was added to the syringes, which were previously heated at 39 °C in the oven, with the help of an automatic dispenser. To obtain the rumen fluid, the rumen contents of two cattle with known history from the slaughterhouse were taken and these fluids were mixed equally and then filtered through a 4-layer cheese bag. Rumen fluid was obtained by ensuring 39°C temperature and CO2 at all stages. Again, the tubes filled with rumen fluid/buffer solution (30 ml) were incubated in a water bath at 39°C and the amounts of gas formed by fermentation were determined at the 3rd, 6th, 12th and 24th hours, respectively. After the 24th hour of incubation, three syringes allocated to detect gas and methane production were removed and cooled to room temperature to stop fermentation.

Gas production: At the end of the incubation at the hours stated above, it was determined in ml by moving the piston in the syringe. To determine net gas production; it was calculated by determining the difference between the amount of gas produced in the prepared samples and the amount of gas produced in the substrate-free (blind) syringe. Three blind syringes were used in each incubation, and the gas produced was calculated by taking the average of the gas produced in these 3 syringes. Obtained gas production results are given as ml/200 mg DM. After measuring the total net gas volume of the samples in 24 hours; the gas produced in the syringe was drawn with a different plastic syringe and injected into the methane detector (Sensor, Europe GmbH, Erkrath, Germany) (to detect the methane rate). The methane ratio of the total gas collected in the syringe was determined as a percentage using a computer program (Goel ve ark., 2008).

In vitro incubations were repeated in three runs, with three replicates per treatment in each run. The

data were analyzed as a randomized complete-block design by using a MIXED procedure of the SPSS Statistics package (IBM SPSS Statistics ver. 22). The statistical model included an overall mean, the fixed effect of treatment, the random effect of incubation run, and the residual error. The linear and quadratic responses were detected with orthogonal contrast. Post hoc analyses were performed using Tukey's test to test pairwise comparisons when there were significant effects of treatments. Standard error of the mean is reported, and the significance was declared at $P < 0.05$.

3. RESULTS AND DISCUSSION

The study was conducted to determine the effects of adding different levels of mealworm (Tenebrio Molitor L.) larvae to alfalfa hay on in vitro gas and methane production. Table 1 shows the gas production, methane production, in vitro organic matter digestibility, and metabolic energy results of mealworm larvae added to alfalfa hay at different rates. As a result of gas production, ml/200 mg DM analysis; it was determined that the highest gas production was in the group in which 1.5% mealworm larvae were added to alfalfa hay (PL<0.05). The lowest gas production was found in the control group. The effect of mealworm larvae in alfalfa hay on methane production was found to be insignificant (P>0.05). In the study, due to the high gas production, organic matter digestibility and metabolizable energy values increased in the treatment groups compared to the control group. According to the results of the study, it was determined that mealworm larvae had higher digestibility compared to alfalfa hay. In previous studies, the in situ ruminal dry matter digestibility of mealworm larvae (24th hour) was determined to be 85.67% (Ayan, 2023). It is known that this is higher than alfalfa. Studies on the use of insects as feed for ruminants are quite limited. In a study, four different insects (TM, Zophobas morio, Alphitobius beziinus and Acheta domesticus) as an alternative feed source in ruminants; intestinal degradability of undigested protein in the rumen was determined in vitro and soybean meal was also used as a reference feed (81-112 g/kg DM content) did not show high ruminal deterioration with a value of 41-76% that the value was lower than that of full-fat soybean flour (Toral ve ark., 2022).

Table 1. Effects of mealworm (Tenebrio Molitor L.) larvae supplementation to alfalfa hay at different levels on in vitro gas and methane production

Treatments	GP	CH4,%	OMD	ME	NEL
C	34.40b	9.30	54.36b	1.48	7.60b
MWL0.5	44.07a	9.53	65.19a	1.72	8.72a
MWL1	44.73a	9.47	65.52a	1.72	8.76a
MWL1.5	46.73a	9.83	67.30a	1.75	9.04a
SEM	1.59	0.13	1.91	0.04	0.22
P Linear	0.012	0.241	0.006	0.040	0.012
P Quadratic	0.036	0.812	0.023	0.188	0.036
P Cubic	0.045	0.562	0.061	0.419	0.045

C: %100 alfalfa hay; MWL0.5: alfalfa +%0.5 yellow meal worm larvae; MWL1: alfalfa+%1 yellow meal worm larvae; MWL1.5: alfalfa +%1.5 yellow meal worm larvae; GP: gas production, ml/200mg; OMD: organic matter digestibility,%; ME: metabolizable energy, MJ/kg DM; NEL: net energy for lactation, MJ/kg DM; SEM: standard error of means.

The small script letters in same column show statistically significance ($p \leq 0.05$).

Studies have reported that insect species cause gas production, methane production and volatile fatty acids like fish meal but less than meals. It has been reported that low degradability in the rumen can be used as an advantage, provided that intestinal digestibility is high (Renna ve ark., 2022). In addition, it is important that methane production, which is a major problem both economically and ecologically (Kaya and Genç, 2022), is low in insect species. In the study conducted by Ahmed and Nishida (2023), it was reported that insects caused less greenhouse gas emissions as a result of being used as animal feed.

CONCLUSIONS

As a result, it was determined that the addition of mealworm larvae to alfalfa hay increased gas production and digestibility. Studies on the effects of mealworm larvae on gas and methane production are quite limited. The findings obtained in this study provide guidance for future in vivo studies and should be supported by further studies.

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