Research Article

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Valorization of dietary edible mushrooms waste: chemical and physical properties, nutrient digestibility, microbial protein synthesis and nitrogen balance in sheep

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Abstract

Background: The optimal use of feed resources must be considered by most livestock farmers. The use of low-cost agricultural by-products and the processing of these materials is one possible solution in this respect. One such compound is edible button mushroom waste (EM), a large amount of which is produced annually in the mushroom production cycle worldwide.

Results: Bulk density 100 of EM was smaller than the other groups. These changes also applied to alfalfa for bulk density, which was higher than the replaced waste. The dry matter solubility of EM was higher than that of alfalfa hay, whereas the ash solubility rate for EM was greater compared to alfalfa. Replacing up to 210 g kg^{-1} alfalfa with EM did not affect the production of purine derivatives, microbial protein, nitrogen excreted in urine and feces, and retained nitrogen, although the organic matter digestibility (OMD) increased, whereas the crude protein digestibility and neutral detergent fiber (NDF) decreased ($P < 0.05$). Fermentation potential, gas production rate, metabolizable energy and short-chain fatty acids were increased. On replacing up to 210 g kg^{-1} alfalfa with EM, the diet OMD increased, whereas the crude protein and NDF digestibility decreased ($P < 0.05$).

Conclusion: EM usage in the experimental diets did not affect the production of purine derivatives, microbial protein, nitrogen excreted in urine and feces, and retained nitrogen. The physical properties, chemical composition and nutritional value of EM, as well as its low cost, show that it can be used as an alternative part of the diet forage in the ruminant's diet.

Keywords: mushroom waste; nitrogen balance; nutritional value; purine derivatives

INTRODUCTION

As a result of the lack of livestock feed resources, it is necessary to identify those factors causing the maximum nutritional efficiency of roughage materials in animal nutrition that can be increasingly used.[1] Approximately 70% of the cost of fattening livestock consists of animal feed and feeding, and the provision of cheap and balanced diets can improve the productivity of this trait.[2] Reducing production costs in the livestock sector is one of the industry's top priorities. For this reason, the optimal use of feed resources must be considered by most livestock farmers. The use of low-cost agricultural by-products and the processing of these materials is one of the proposed solutions for this purpose.[3] Some plant sources can be used as livestock and poultry feed. However, because of a lack of knowledge and a lack of information about how to use from these unknown sources, it is not recommended and cannot be offered for animals. Some of these wastes are used in traditional and informal livestock farms, the use of which in the diet of livestock requires special attention with respect to agricultural waste.[4] By-products and processed feeds have long been used in animal nutrition and have several advantages. First, the nutrition of these products makes the animal less dependent on grains and feeds that can be used by humans. Second, additional costs eliminate the direction of management programs, and the consumption of such products becomes more important as the price of common feeds increases.[5] In many cases, the use of these products has many problems because of their biological instability, pathogenic nature, high humidity, rapid spoilage and high enzymatic activity.[6] One of these compounds is button mushroom compost waste, a large volume of which is produced annually in the mushroom production

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cycle in Iran and the world.[7] Edible mushrooms are among the saprophytic fungi and belong to the category of basidiomycetes.[8] Mushrooms are used as a feed source.[8] There are small or large industries that grow edible mushrooms in the world and Iran, producing these mushrooms for direct feed consumption.[7] One of the most famous species of edible mushroom grown almost everywhere worldwide and popular with consumers is the button mushroom *Agaricus bisporus*.[9] In mushroom cultivation units, the materials obtained as waste include large amounts that are damaged, small and deformed, with a long base and tail of the fungus. In most cases, these wastes are discarded or, in a few cases, they enter the composting process in small volumes. Recently, it has been delivered to the essential oil industry for the preparation of mushroom essential oil.[8] These wastes are rich in nitrogen, cellulose and minerals.[9] Edible button mushroom waste causes environmental pollution as a result of high humidity and low maintenance.[10] To increase the shelf life of this feed, silage and drying methods can be used and also, before use, pesticides, mycotoxins, heavy metals and anti-nutritional agents should be considered in terms of pesticides.[7,11] Few studies have reported on the use of this waste in animal feed.[9,12,13] They have reported the use of fungal waste and studied its fiber effects. The by-product after harvest of edible mushrooms comprises mushroom residues and compost, which, in addition to having a large and diverse group of microorganisms, contains a wide range of active extracellular enzymes that digest the lignocellulosic compounds such as wheat straw.[6] Even if research has been conducted regarding edible button mushroom waste in ruminants, there is still not much information available and, today, edible button mushroom waste has not been officially considered in animal nutrition, including waste, and it is completely discarded. In the present study, an attempt has been made to evaluate the nutritional value of these wastes with respect to preventing environmental pollution and replacing the forage part of the sheep's diet. As a result of the shortage of animal feed and the low cost of this by-product, the present study was proposed and implemented aiming to introduce edible button mushroom waste for use in animal feed, especially in ruminants.

MATERIALS AND METHODS

The research protocol in the present study, in cases of working with animals, was conducted in accordance with the recommended platform of the Committee on Livestock Care of the University of Isfahan.[14]

Animals, experimental design and treatments

The ingredients and chemical composition of the dietary treatments based on dry matter (DM) are shown in Table 1. Four diets, based on the nutrient requirements recommended by the National Research Council and the Small Ruminant Nutrition System (SRANS) software (version 24, Texas, USA) for four animals kept in a metabolic cage, were adjusted and fed according to maintenance needs.[15,16] The animals were managed according to the standard operating procedures of the research station of Mohaghegh Ardabili University and guidelines set by the Iranian Council of Animal Care.[14] The experiment was performed with four male Moghani sheep with a mean ± SD weight of 46 ± 2 kg in four periods each of 20 days, of which 15 days were adaptation and 5 days were sampling. Each sheep was housed in a metabolic cage (0.8 × 1.05 m) and each site was equipped with a separate manger and scavenger. The experimental design was a 4 × 4 Latin square design in one of the four dietary treatment groups. General health control and vaccination with enterotoxaemia vaccine (Brilliant Biopharma Private Ltd, Pashamylaram, India; 2 mL subcutaneous per sheep) and anthelmintic (Albendazole BZD; Valbazen, Pfizer Animal Health, Group, Lee's Summit, MO, USA; 5 mg kg^{-1} bodyweight, with a second repetition after 2 weeks) drugs were also administered at the beginning of the experiment. Feeding was carried out twice a day at 08.00 h and 16.00 h. The weight of the feed and its residues were measured daily.

Chemical composition and physical properties

The chemical compounds of samples for CP, EE and ash were measured by conventional methods (AOAC).[17] Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured as described by Van Soest *et al.*[18] using the filter bag system (ANKOM Technology, Macedon, NY, USA). The physical properties of the edible specimens in the present study have been modeled by making some minor changes to the method of Giger-Rordin.[19] Each oral sample was tested with five replications. Mass density was measured in three modes. (i) Mass density of 50 [bulk density (BD_{50})]: A 100-mL calibrated cylinder (with an internal diameter of 2.5 cm) was used for measurement, so that the milled edible sample (with a 1-mm sieve) was poured into it up to a volume of 50 mL, then, with the use of a vortex device, it was shaken for 15 s at 220 × g. The weight and volume of the material were recorded inside the graduated cylinder. The sample weight in the volume of 50 mL was calculated using proportionality and a mass density of 50 was reported. (ii) Mass densities of 50 and 100 (BD_{50}): For the oral sample in the calibrated cylinder at the stage of measuring the mass density of 50, the oral sample was poured again to reach a volume of 100 mL, and then shaken for 10 s as described in the previous step. The volume and weight of the oral sample were recorded in a calibrated cylinder and the results were calculated as in the previous step and reported as a mass density of 50 and 100. (iii) Mass density of 100 (BD_{100}): At this stage, an oral sample of up to 100 mL was poured into a graduated cylinder. This was shaken as before for 5 s and the calculations performed as described above. The volume and weight of the oral sample were recorded and the results were reported as a mass density of 100.

Apparent digestibility and gas production parameters

To determine the apparent digestibility of feed and nutrients throughout the gastrointestinal tract, acid-insoluble ash (AIA) was used as an internal marker. The amount of AIA was estimated as described by method Van Keulen and Young.[20] In brief, the dietary and fecal samples were also analyzed for AIA using a modification of the 2 mol L^{-1} HCl procedure. The total fecal collection DM digestibility (DMD) was calculated as (dietary intake–fecal output) × 100)/dietary intake. The AIA DMD was calculated as the ratio of AIA in the feed and feces[21]:

$$\%\text{AIA DMD} = 100 - 100 \times \frac{\%\text{marker in feed}}{\%\text{marker in feces}} \times \frac{\%\text{nutrient in feces}}{\%\text{nutrient in feed}} \quad (1)$$

The *in vitro* gas test method was performed according to Menke and Steingass.[22] The volumes of gas were measured at incubation times of 3, 6, 12, 18, 24, 48, 48, 72 and 96 h for 200 mg of dry sample in each syringe with three replications and two runs. The three ruminally fistulated Maghani sheep (bodyweight = 50 ± 0.8 kg) were used for collecting rumen fluid before the morning feed. The rations of fistulated animals consisted of alfalfa hay and concentrates as a total mixed ration (1:1, wt/wt) and were fed in two equal

quantities (approximately 5% refusal) at two meals daily. The strainer pole was carefully inserted into the bottom of the rumen of the fistulated sheep. Then, the plastic sample tube was inserted into the strainer pole. The rumen fluid was drawn slowly into attached the syringe to the outside end of the tube. The mixed equal volume of rumen liquid obtained by the three sheep was filtered by four layers of cheesecloth within a preheated thermos and taken to the research center. Each of the running procedures of rumen liquid was carried out under continuous purging of CO_2.

The syringes in this technique are filled with 30 mL of medium (10 mL of ruminal fluid + 20 mL of McDougall buffer). Buffer contained (in the order added) 500 mL of H_2O, 0.1 mL of solution A, 200 mL of solution B, 200 mL of solution C, 1 mL of resazurin (0.1%, w/v) solution D and 40 mL of reduction solution E. This combination was then kept under CO_2 at 39 °C in a water bath and agitated using a magnetic stirrer. Solution A consisted of 13.2 g of $CuCl_2H_2O$, 10.0 g of $MnCl_2.4H_2O$, 1.0 g of $CoC1_2.6H_2O$ and 8.0 g of $FeCl_2.6H_2O$, made up to 100 mL with water. Solution B consisted of 35 g of $NaHCO_3$ and 4 g of NH_4HCO_3 made up to 1000 mL with water. Solution C consisted of 5.7 g of Na_2HPO_4, 6.2 g of KH_2PO_4 and 0.6 g of $MgSO_4.7H_2O$ made up to 1000 mL with water. Solution D consisted of 0.5 g of resazurin, made up to 100 mL with water. Solution E is the reduction solution consisting of 95 mL of H_2O, 4 mL of 1 mol L^{-1} NaOH and 625 mg of $Na_2S.9H_2O$. Each sample (200 ± 1 mg) was accurately weighed in three replicates into 100-mL glass syringes fitted with plungers and incubated in a continuous rotary shaker. Negative controls (ruminal fluid plus buffer alone) were also incubated in three replications in the run. Equation (2) of France *et al.*[23] was used to determine the extraction points of gas production:

$$G = A\left\{1-\exp^{\left[-c\,(t-L)-d\left(\sqrt{t}-\sqrt{L}\right)\right]}\right\} \quad (2)$$

where G is equal to the accumulation of gas produced per unit of time, A is equal to the total amount of gas produced (mL), c is equal to a constant rate of gas production (mL h^{-1}), d is equal to a constant rate of gas production (mL at h1/2;), L is equal to the lag time, and t and $t_{½}$ time are equal to half of the total gas production time is cumulative.

The Menke and Steingass eqns (3) and (4)[22] were used to estimate metabolizable energy, net energy lactation and digestible organic matter, and eqn (5) of Getachew *et al.*[24] was used to estimate short-chain fatty acids:

$$\mathrm{ME}\left(\mathrm{MJ\,kg^{-1}\,DM}\right) = 1.06 + 0.1570\mathrm{GP} + 0.0084\mathrm{CP} + 0.0220\mathrm{EE} - 0.0081\mathrm{CA} \quad (3)$$

$$\mathrm{OMD}\,(\mathrm{DM\%}) = 9 + 0.99\mathrm{GP} + 0.0595\mathrm{CP} + 0.018\mathrm{CA} \quad (4)$$

$$\mathrm{SCFA}\left(\mathrm{mmol\,200\,mg^{-1}\,DM}\right) = 0.0222\mathrm{GP} - 0.00425 \quad (5)$$

where ME is metabolizable energy (MJ kg^{-1} DM), GP gas is 24-h net gas production (mL g^{-1} DM), CP is crude protein (DM %) and EE is crude fat (DM %), CA is ash in g 100 g^{-1} DM, OMD is organic matter digestibility (g 100 g^{-1} DM), and SCFA is short chain fatty acid (mmoL).

Microbial protein production and nitrogen balance

At the end of each period, the animals were collected after the adaptation period of urine and feces. Urine collection containers contained 100 mL of 10% sulfuric acid. Some 70 mL of urine was collected daily and transferred to containers and stored at −20 ° C. Urinary uric acid was measured using a Pars Azmoun and Allantoin Urine Kit (Pars Azmoon Co., Tehran, Iran) in accordance with the method of Chen and Gomez.[25] The amount of microbial protein made from the total excreted purine bases was calculated. The efficiency of microbial protein production was obtained from the ratio of microbial protein produced to the DM consumed per day. To determine the efficiency of microbial protein production in terms of degraded organic matter in the rumen, the effective degradability of organic matter in the experimental diets was calculated and, finally, the value of microbial protein produced per day was measured. Nitrogen balance was calculated by deducting total nitrogen excreted in the urine and feces from the nitrogen intake from the feed.

Table 1. Ingredient and chemical composition of dietary treatments based on DM[a]

Items	EM0	EM7	EM14	EM21
Ingredients (g kg^{-1} DM)				
Hay alfalfa	300.0	210.0	140.0	70.0
Edible button mushrooms waste	0.0	70.0	140.0	210.0
Wheat straw	292.5	320.0	325.0	342.5
Barley grain	300.0	300.0	305.0	310.0
Wheat bran	45.0	40.0	45.0	30.0
Soybean meal	52.5	50.0	35.0	27.5
Edible salt	5.0	5.0	5.0	5.0
Mineral supplement and vitamin[b]	5.0	5.0	5.0	5.0
Chemical composition (g kg^{-1} DM)				
Dry matter	892.0	901.8	905.2	910.2
Crude protein	122.0	123.7	123.3	123.1
Metabolisable energy (Mcal kg^{-1})	2.3	2.4	2.5	2.6
Neutral detergent fiber	488.2	492.7	491.6	493.7
Crude ash	76.6	87.5	99.2	111.2

[a] Edible button mushrooms waste (EM) dietary inclusion at 0 (EM0), 70 (EM7), 140 (EM14) and 210 g kg^{-1} (EM21), replacing alfalfa hay by 0 (control), 70, 140 and 210 g kg^{-1}, respectively.
[b] Each kilogram of the supplement contains vitamin A (500 000 IU), vitamin D3 (100 000 IU), vitamin E (100 mg), phosphorus (20 g), sodium (50 g), magnesium (20 g), iron (3 g), manganese (2 g), zinc (3 g), copper (280 mg), cobalt (100 mg), iodine (100 mg) and selenium (4 mg).

Table 2. Chemical composition, physical properties (solubility and mass density), *in vitro* gas production and nutritional parameters of edible button mushroom waste and alfalfa hay

Items	Edible button mushroom waste	Alfalfa hay
Dry matter (DM)	238.6 ± 5.0	–
Crude protein	214.0 ± 4.0	–
Ether extract	37.5 ± 0.50	–
Organic matter	709.3 ± 9.0	–
Neutral detergent fiber	393.3 ± 6.0	–
Acid detergent fiber	255.4 ± 4.0	–
Lignin	216.4 ± 3.0	–
Non-fiber carbohydrates[a]	64.5 ± 0.4	–
Carbohydrates total	457.8 ± 5.5	–
Dry matter solubility (g kg^{-1} DM)	531.5 ± 6.0	375.3 ± 9.8
Organic matter solubility (g kg^{-1} DM)	543.9 ± 5.8	322.4 ± 12.2
Organic matter in DM solubility (g kg^{-1})	676.5 ± 4.1	295.4 ± 5.0
Mass density (g mL^{-1}) BD_{50}[b]	0.353 ± 0.01	0.361 ± 0.04
Mass density (g mL^{-1}) $BD_{50,100}$[c]	0.363 ± 0.01	0.377 ± 0.01
Mass density (g mL^{-1}) BD_{100}[d]	0.319 ± 0.01	0.369 ± 0.01
Fermentation potential (mL g^{-1} DM)	531.5 ± 7.0	–
Gas production rate (unit h^{-1})	0.07 ± 0.01	–
Lag phase (h)	0.3 ± 0.08	–
24-h gas production (mL 200 mg^{-1} DM)	90.2 ± 0.9	–
Metabolizable energy (MJ kg^{-1} DM)	15.5 ± 0.2	–
Net energy lactation (MJ kg^{-1} DM)	10.1 ± 0.1	–
Digestible organic matter (g kg^{-1} DM)	117.1 ± 2.0	–
Short-chain fatty acids (mmol 200 mg^{-1} DM)	2.0 ± 0.01	–

[a] Non-fiber carbohydrates = 100 − (neutral detergent fiber + crude protein + crude fat + ash.
[b] Bulk density: Filling the graduated cylinder to a volume of 50 mm in one step.
[c] Bulk density: Filling the graduated cylinder up to a volume of 50 mm and up to a volume of 100 mm.
[d] Bulk density: Filling the graduated cylinder to a volume of 100 mm in one step.

Statistical analysis

The data were analyzed in a Latin square design with a general linear model procedure. Response variables during the treatment period were compared using orthogonal contrasts with SAS, version 9.1.[26] In this plan, the statistical model used was:

$$Y_{ikjm} = \mu + R_i + C_k + T_j + E_{ikjm}$$

where Y_{ikjm} is the indicator of each observation in the experiment, μ is the mean of the total population that is examined through the samples with zero assumption, R_i is the row effect, C_k is the column effect, T_j is the treatment effect and E_{ikjm} is the random error effect of the test. The multiple comparison analysis in the present study was performed using Duncan's test at an error level of 0.05.[26]

RESULTS AND DISCUSSION

Chemical composition and physical properties

The results regarding chemical composition, DM solubility and mass density of edible button mushroom waste (i.e. EM) are shown in Table 2. It was found that BD_{100} was smaller compared to the other groups. These changes also applied to alfalfa, which was higher than the replaced waste.

Based on the results reported by Pazoki *et al.*,[27] the crude protein content of edible button mushrooms was 267 g kg^{-1} DM. According to research conducted by Tsai *et al.*,[28] the protein content was 213.7 g kg^{-1} DM, which was described as the global standard, and this indicates that the protein content of the present study is acceptable with respect to the global standard. Differences in chemical compounds result in different nutritional values because chemical compounds (Table 2) are the most important indicators of the nutritional value of feed.[24] Some sources reported moisture in fresh mushrooms in the range 950–850 g kg^{-1}.[29] The fat content of edible mushrooms was higher ($P < 0.05$) than that of oyster mushrooms. Mushrooms are known to have low-fat levels.[30] Previous studies have reported that there are differences for different species of mushrooms because the moisture content of the mushrooms is affected by humidity, temperature of the cultivation and storage rooms.[31] The crude protein of these wastes was slightly higher than that reported by Rai *et al.*[31] The chemical composition of mushrooms is very different in terms of type, variety, processing method and production.[32] Maheri Sis *et al.*[33] noted that the different chemical compounds in the wastes tested in different studies differ as a result of raw materials, cultivation conditions, DM content, impurity content and different measurement methods and, in studies by Dehghan *et al.*[34] and Telkozadeh *et al.*,[35] the chemical composition of button mushrooms was reported and compared with several by-products used in animal nutrition (Table 2). No research was conducted on the use of edible mushroom waste, although much research has been carried out on the compost mushroom or other agricultural waste. In their research on mushroom compost waste, Bakshi and Langar[36] and Riahi *et al.*[37] observed that the amount of crude protein in these wastes was higher than that of ordinary straw and alfalfa, and the reason for this was related to the use of nitrogen fertilizer during the preparation of the compost and the remnants of edible mushroom waste. The results of Bakshi and Langar[36] show that the protein content of mushroom compost was higher than that of ordinary straw and, according to the results obtained, the energy level was low because of the high ash content. Yamakawa *et al.*[38] reported that the most changes in compost straw compared to raw straw reduced organic matter, whereas the amount of crude protein increased. As a result of the removal of soil in the silage, mushroom compost wastes showed a reduced ($P < 0.05$) percentage of crude protein and crude ash because of the loss of part of the soluble nitrogenous material and soil in the compost mushroom waste at the time of washing, respectively.[10] The soil in these materials was considered to be responsible for an increase in the amount of raw ash and a consequent decrease in the percentage of organic matter in materials that are contaminated or mixed with soil.[39] As remnants of the edible button mushroom used in this experiment have a higher ash content than the other

agricultural products reported in Dehghan *et al.*,[34] Telkozadeh *et al.*[35] and Kalvandi *et al.*,[7] and the reason for this, according to research by Behgar[40], is the high potassium content of these wastes. Giger-Rodin[19] reported that materials with low mass densities usually had low cell walls, and it was generally concluded that fodder would have low mass densities and also that grains and cereals would have high mass densities. The results reported by Singh and Narang[41] show that, by breaking down the cell wall of nutrients, their mass density increased because of the reduction of the void space in cellulose, and they also stated that the capacity between mass density water retention is associated with voluntary feed intake. Tahmasebi *et al.*[42], researching walnut green skin in a pure and processed form with *Neurospora sitophila* fungi, observed that the mass density for these two treatments and in two cases, BD_{50} and BD_{100}, was 3.54 *versus* 3.50 mL^{-1} and 1.99 versus 1.93 mL^{-1}, respectively, such that the reported values did not differ from each other and were less than in the present study. The observations made by Golchin Goldoni *et al.*[43] during a study on long alfalfa hay as powdered and acid processed or untreated from each, stated that the mass density of alfalfa-containing diets was higher than that of alfalfa rations. They were generally processed and pure diets did not differ with respect to physical characteristics. The data regarding DM solubility amount for edible mushroom waste was higher than the amount for alfalfa, whereas the rate of ash solubility for edible mushroom waste was greater compared to that for alfalfa. Yalchi *et al.*[44] stated that sugarcane molasses have the highest and most complete (100%) solubility among all of feeds used. Giger-Rordin[19] reported that there were differences in the solubility of feedstuffs and according to studies coducted with different feeds, it was found that citrus pulp, as a result of its high pectin content and low insoluble fiber in neutral detergents, has the highest solubility, which is slightly different from the value obtained for edible button mushrooms in the present study.

Apparent digestibility and *in vitro* gas production

On increasing the substitution of up to 210 g kg^{-1} alfalfa hay with edible mushroom waste, the organic matter digestibility increased ($P < 0.05$), although the digestibility of crude protein and NDF decreased ($P < 0.05$). However, the crude protein digestibility showed a linear decrease and quadratic effects, whereas NDF digestibility demonstrated decreased linear, quadratic and cubic behaviour ($P < 0.05$). Subsequently, the full models contained decreasing linear and quadratic effects ($P < 0.05$) for all dietary digestibility variables except for ether extract. It was also observed that organic matter digestibility increased ($P < 0.05$) with respect to linear, quadratic and cubic effects of increasing EM inclusion in the diets, although linear and quadratic effects were reduced ($P < 0.05$), the digestibility of crude protein, linear, quadratic and cubic effects were reduced ($P < 0.05$) and NDF digestibility and linear effects reduced the digestibility of ADF (Table 3).

It was found that determining the digestibility of feed was one of the most important factors with respect to determining some of the nutrients available to the animal.[21,45] Van Soest[21] stated that the rate of digestibility of diets varies in animals and is influenced by the species of the animal, as well as the amount and composition of the diet. The digestibility of nutrients increases with increasing feed intake, normal fermentation of rumen and dietary supplementation.[46] According to Titgemeyer *et al.*,[47] cell wall degradability is related more to its structure than the rumen medium or shelf life. In the results reported by Pir-Mohammadi and Azizi,[48] silage apple pulp samples had a high cell wall and were less digestible in terms of DM and organic matter. Tahmasebi *et al.*[42] stated that the digestibility of DM of feedstuffs has a relatively high negative relationship with water storage capacity. It also suggested that the use of edible button mushrooms reduced the rate of digestion in the rumen environment and may have improved the performance of ruminal microbes. Guimaraes *et al.*[49] attributed the decline in digestibility to DM, organic matter and the cell wall of feed by adding waste to the diet to various factors, including the physicochemical nature, type and amount of structural carbohydrates, and the presence of tannins. The dense tannins of the binding sites affect the enzymatic activity of the ruminal microbial ecosystem, followed by the ruminal degradation of different parts of the feed. NDF digestion through binding to bacterial enzymes or the formation of indigestible complexes with cell wall carbohydrates may also be reduced by tannins.[50]

The results obtained using *in vitro* gas production for metabolic energy, digestible organic matter and short-chain fatty acids in the present study were higher than the results of previous investigations of the use of edible mushroom compost at amounts of 8.95 MJ kg^{-1}, 560 g kg^{-1} and 0.812 mmol 200 mg^{-1} DM, respectively.[51] The increase in the gas produced in the present study can be attributed to a high digestibility because there is a positive correlation between digestibility and gas production.[24] Taghizadeh *et al.*[52] observed that the more hemicellulose each cell is, the more gas it produces. Blummel and Ørskov[53] reported that feeds with high protein content

Table 3. The effect of different levels of edible button mushroom waste (EM)[a] on nutrient digestibility in Moghani sheep males[†]

						P-value			
Digestibility (g kg^{-1} DM)	EM0	EM7	EM14	EM21	SEM[‡]	Model	Linear	Quadratic	Cubic
Dry matter	616.7 a	602.8 b	579.1 c	598.9 b	3.60	0.003	0.003	0.003	0.017
Organic matter	641.7b	649.5 b	638.3 b	664.9 a	3.30	0.003	0.007	0.028	0.008
Crude protein	703.9 a	668.2 b	651.0 b	663.6 b	4.90	0.002	0.001	0.003	0.616
Ether extract	679.2	663.0	676.2	681.0	33.20	0.631	0.904	0.764	0.808
Neutral detergent fiber	588.1 a	582.5 a	529.2 c	566.9 b	3.10	0.001	0.001	0.001	0.001
Acid detergent fiber	365.2 a	392.6 a	178.2 b	251.1 b	22.00	0.011	0.001	0.341	0.001

[†] Edible button mushrooms waste (EM) dietary inclusion at 0 (EM0), 70 (EM7), 140 (EM14) and 210 g kg^{-1} (EM21), replacing alfalfa hay by 0 (control), 70, 140 and 210 g kg^{-1}, respectively.
[‡] Standard error of the mean.
Different lowercase letters in each row indicate a significant difference in the error level of 0.05.

have a lower energy value in the gas production method relative to its true extent because carbon dioxide remains in the liquid and does not escape. Also, Larbi *et al.*[54] expressed a positive relationship between the amount of crude protein and gas production, which is consistent with the results of the present study. It should be noted that results from other studies for *in vitro* gas production of edible mushrooms waste are not available. Therefore, the parameters of gas production of edible mushrooms waste have been compared with other wastes. Nakhaei *et al.*,[55] during their research on the processing of reed fodder with oyster mushrooms (*Pleurotus ostreatus*), observed that the highest amount of gas production in reed fodder was the second mushroom harvest. The results of their study were less consistent with the results of the present study regarding button mushroom waste. A study by Okano *et al.*[56] on oyster-processed bagasse reported similar results to those of Nakhaei *et al.*[55] The reason for the increase in gas production with the development of mushroom growth is related to the effect of fungal enzymes in the degradation of structural parts of bagasse fodder and the increase of soluble compounds. The important relationship between ADF and NDF with the proportion and volume of gas produced was observed by Hadi *et al.*,[57] these results are related to the reduction of the cell wall and the insoluble part of the test material. In general, the results reported by Behgar[40] are different from the results of the present study and the amount of gas produced was less than that observed in the present study.

Microbial nitrogen produced, purine derivatives and nitrogen balance

So far, there have been no studies on the effect of different levels of edible button mushroom waste on microbial production in sheep, nor its estimation based on purine derivatives.

Total purine derivatives such as allantoin, acid uric, xanthine and hypoxanthine, and even the total absorbed or excreted purine derivatives, did not differ ($P > 0.05$). Microbial protein production was not different ($P > 0.05$) between experimental treatments, ranging between 38.4 and 41.2 g day^{-1}. The total excretion of purine derivatives and the absorbed purine derivatives did not differ ($P > 0.05$) between treatments. However, no difference was observed between the experimental treatments with respect to the synthesis of microbial protein (Table 4).

Nitrogen consumed, urinary nitrogen excretion, total excreted nitrogen and nitrogen retention did not show any differences ($P > 0.05$) with the addition to the residual amount of edible button mushrooms in the diet by up to 210 g kg^{-1} ($P > 0.05$) (Table 5).

In the study by Tahmasebi *et al.*[58], the reported amount of excretory allantoin was calculated to be 0.57, 0.71 and 0.90 mmol mL^{-1} (body weight) $BW^{0.75}$ with increasing feed intake (70%, 85%, 100%, respectively). There was an increase in the amount of feed consumed and the amount of excreted allantoin also increased, which does not correspond with the results of the present study. Tahmasebi *et al.*[46] observed that mean excretory allantoin in sheep was affected by the experimental diet (alfalfa silage feeding with

Table 4. The effect of dietary edible button mushrooms waste (EM)[a] on purine derivatives and microbial protein production in sheep[a]

						P-value			
Items	EM0	EM7	EM14	EM21	SEM[b]	Model	Linear	Quadratic	Cubic
Allantoin (mmol day^{-1})	5.3	6.0	6.2	6.2	0.76	0.342	0.691	0.913	0.994
Xanthine and hypoxanthine (mmol day^{-1})	1.4	1.2	1.2	1.1	0.30	0.412	0.794	0.904	0.882
Uric acid (mmol day^{-1})	1.0	0.8	0.9	0.8	0.13	0.024	0.892	0.633	0.334
Total excreted purine derivatives (mmol day^{-1})	7.7	8.0	8.3	8.1	0.60	0.464	0.741	0.762	0.873
Total absorbed purine derivatives (mmol day^{-1})	8.5	8.8	9.1	8.8	0.71	0.451	0.742	0.754	0.883
Microbial nitrogen production (g day^{-1})	6.1	6.4	6.6	6.4	0.52	0.461	0.732	0.763	0.874
Microbial protein production (g day^{-1})	38.4	39.8	41.2	40.1	3.25	0.452	0.734	0.752	0.883

[a] Edible button mushrooms waste (EM) dietary inclusion at 0, (EM0), 70 (EM7), 140 (EM14) and 210 g kg^{-1} (EM21), replacing alfalfa hay by 0 (control), 70, 140 and 210 g kg^{-1}, respectively.
[b] Standard error of the mean.

Table 5. The effect of different levels of edible button mushroom waste (EM)[a] on nitrogen balance and retention in sheep[a]

						P-value			
	EM0	EM7	EM14	EM21	SEM[b]	Model	Linear	Quadratic	Cubic
Nitrogen intake (g day^{-1})	23.9	22.4	22.7	22.8	0.28	0.140	0.052	0.034	0.206
Nitrogen excreted in the urine (g day^{-1})	9.6	8.9	7.6	8.1	1.09	0.436	0.282	0.620	0.647
Nitrogen excreted in the feces (g day^{-1})	7.1	7.5	7.9	7.7	0.17	0.058	0.027	0.107	0.282
Total nitrogen excreted in urine and feces (g day^{-1})	16.2	16.3	15.5	15.7	1.06	0.497	0.492	0.829	0.774
Total retained nitrogen (g day^{-1})	7.3	6.2	7.1	7.1	1.13	0.571	0.936	0.654	0.547
Nitrogen retention efficiency (%)	30.4	27.2	31.3	31.1	4.74	0.537	0.778	0.772	0.605

[a] Edible button mushrooms waste (EM) dietary inclusion at 0 (EM0), 70 (EM7), 140 (EM14) and 210 g kg^{-1} (EM21), replacing alfalfa hay by 0 (control), 70, 140 and 210 g kg^{-1}, respectively.
[b] Standard error of the mean.

different amounts of waste dates) and the reported numbers for the studied treatments were 6.49, 7.29, 7.83 and 8.15 mmol day^{-1}, respectively), which is different from and higher than the results of the present study. The increase in allantoin excretion was accompanied by an increase in the level of waste dates in the experimental diets, possibly as a result of an increase in the amount of easily digestible carbohydrates for ruminal microorganisms and the simultaneous production of high energy and protein in the rumen. As the feed intake increases, the growth and proliferation of ruminal microorganisms increases because of the availability of energy, which in turn increases microbial protein production. The nucleic acids of ruminal microbes decompose when they reach the gut, and purine and pyrimidine nucleosides are broken down by purine oxidase enzymes to purine derivatives.[59] A study by Johnson *et al.*[60] found that there was a linear relationship between urinary excretory allantoin and digestible DM and digestible organic matter, which was used to demonstrate a strong association between urinary excretory allantoin and microbial nitrogen flow. Because the amount of digestible DM and digestible organic matter increased, the amount of microbial protein introduced into the small intestine increased. The uric acid concentration in sheep fed the experimental diet was affected by the value of waste dates associated with alfalfa silage, and the reported numbers for the treatments were 0.22, 0.23, 0.23 and 0.25 mmol day^{-1}). These figures were lower than the value reported in the present study. The production of microbial protein in the rumen is measured by the amount of microbial nucleic acid entering the small intestine. An increased discharge of purine catabolic by-products in the urine indicates that more microbial nucleic acids have entered the small intestine.[61] According to the results obtained, the mean of purine derivatives (absorbed and excreted) did not change with increasing compost waste of button mushrooms in sheep's diet and, in addition, the obtained results were consistent with the results of Tahmasebi *et al.*[46] who found that the amount of nitrogen and microbial protein produced was not affected by the diet. Chen *et al.*[61] stated, based on their research, that, if the ratio of concentrate increased, the synthesized microbial protein increased. Because there is sufficient nitrogen in the rumen, the synthesis of microbial protein is a function of the ability of microorganisms to access energy. Chamberlain *et al.*,[62] based on the results of their research on pure carbohydrates (sucrose and starch), stated that pure carbohydrates increase the retention of nitrogen in the rumen and the synthesis of microbial protein. Excessive microbial protein in sheep fed an experimental diet indicates that diets using a lot of waste are highly effective with respect to preventing the production of ammonia and using it to make microbial protein. Amlan and Jyotisna[63] reported that, in general, the transfer of nitrogen excretion from the urine to the feces increases nitrogen retention and improves nitrogen utilization efficiency. Therefore, reducing nitrogen excretion in the urine can reduce the release of ammonia and nitrous oxide into the atmosphere.[64] The first way of reducing nitrogen excretion in the urine[65] or feces, or both[66] is to reduce nitrogen intake in the diets. This solution is achievable when it does not significantly reduce the yield of livestock; another way of reducing nitrogen excretion in livestock is to improve the efficiency of nitrogen consumption.[67] Nitrogen metabolism in the rumen is known to be the most important factor for improving nitrogen consumption.[68]

CONCLUSIONS

The edible button mushrooms is an average quality feed and can be used in situations where the farmer is faced with a limited supply of feed to meet animal nutritional requirements. Because of its cheapness and excellent nutritional value, this by-product can be recommended for consumption in animal feed by replacing up to 21% of alfalfa hay, especially in ruminants. This residue can be considered as a by-product of fibro-protein. Replacing these wastes by up to 21% with a portion of alfalfa fodder in the sheep's diet, at the same time as increasing the organic matter digestibility from 641.70 to 663.60 g kg^{-1} DM, did not affect purine derivatives and microbial protein production.

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CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

ETHICAL STATEMENT

This study was approved by the Isfahan University of Technology, Local Ethics Committee, Iran.

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