In vitro and in sacco determining the nutritive value of button mushroom stipe and its application in growing lambs diet

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ABSTRACT
Feed shortage is one of the major challenges in livestock production systems in the arid and semi-arid regions of the world. Hence, wastes of nutritional value from the agro-alimentary industry could be considered as by-product feedstuffs, reducing diet costs in these regions. The current study aimed at determining in vitro and in sacco nutritive value of button mushroom stipe (BMS) and investigating its potential in growing lambs diet. For this purpose, three experiments (Exp.) were conducted. Ruminal degradability characteristics, and gas production kinetics, and energetic value of BMS compared with alfalfa were determined by the nylon bag and gas production techniques in Exp.1 and Exp.2, respectively. In Exp.3, 24 Mehraban growing lambs were assigned randomly to one of the three dietary treatments: (1) the basal ration without BMS (CTRL); (2) and (3) inclusion of 10 and 20% (DM basis) of BMS in the basal ration (BMS10 and BMS20, respectively), to determine their growth performance and the rations digestibility. The soluble (a) and slowly degradable (b) fractions, and degradation rate of 'b' fraction of the BMS crude protein (CP) was 43, 59%, and 0.034, respectively. The metabolisable energy (ME) content of BMS was 20.6% lower than that in alfalfa. The inclusion of BMS in the ration of lambs did not affect their growth performance, but reduced diet cost by 7.2 and 14.5% in BMS10 and BMS20, respectively. The BMS tended to increase (p = .089) the blood total protein in lambs fed BMS10 and tended to decrease (p = .07) alkaline phosphatase (ALP) in BMS-based diets. These results reveal that BMS has a slow ruminal degradability and fermentation that can reduce diet cost by partially replacing conventional feedstuffs in the ruminant diet without adversely affecting their health and performance.

HIGHLIGHTS
- The button mushroom stipe (BMS) by-product has relatively high crude protein and acceptable energy contents with a slow ruminal degradability.
- Including BMS in the diet of growing lambs reduced diet costs, while improving fairly their growth performance, implying that BMS can be considered as a cost-effective by-product feedstuff in a ruminant diet.
- The inclusion of BMS in the diet of lambs decreased ruminal ammonia production, which can reduce the environmental impact of ruminant production systems.

Introduction
Edible mushrooms are one group of macro-fungi with a fruiting body, which has been constituted an integral part of the human diet and consumed for food during antiquity (Wani et al. 2010). However, mushrooms have received growing interest over the last few decades because of discovered evidence of their several beneficial effects on human health (Singh et al. 2014; Rathore et al. 2017). Hence, the production of edible mushrooms has been doubled during the last decade, attaining an annual yield of around 10 million tons worldwide (FAO 2016). Correspondingly, a large quantity of their wastes is also produced annually from the mushroom industry that should be disposed of (Nasiri et al. 2013; Lopusiewicz 2018; Baziuon et al. 2020). However, some of these wastes of nutritional value can be considered as by-products that have the potentials to be used as new feedstuffs for ruminants.

The spent mushroom substrate is one of the by-products from commercial production of edible mushrooms with a higher CP and soluble cell-wall contents than the original lignocellulose biomass, which has
been used in ruminant diet (Van Kuijk et al. 2015). Mushroom stipe is another by-product of the mushroom production industry, which is the lower part of the mushroom stem remaining after harvesting the mushroom crop and separating the cape and upper part of the stem from the fruit body.

Edible mushrooms contain a wide range of bioactive compounds, such as mono-, di-, and oligosaccharides, phenolic compounds, ergosterols, lectins, and carotenoids (Karaman et al. 2010; Liu et al. 2013; Singh et al. 2014; Muszyńska et al. 2017). Hence, in most of the previous experiments conducted on animal nutrition, mushrooms have been considered as feed additives, especially with the potential of replacing antibiotic growth promotors. In this context, many edible mushrooms including *Agaricus bisporus*, *Pleurotus ostreatus*, *Lentinula edodes*, and *Hericium caput-medusae* have been reported to improve the performance and antioxidiant capacity of broiler and turkey chickens (Guo et al. 2004; Giannenas et al. 2010; Giannenas et al. 2011; Shang et al. 2014). Moreover, mycelia and spent mushroom of *Cordyceps* fungi species have been reported as a news feed additive in ruminants with the capacity of improving the rumen fermentation, total tract digestibility, and animal health status (Yeo et al. 2011; Kim et al. 2014; Chanjula and Cherdthong 2018). Among edible mushrooms, white button mushroom is the most preferred and cultivated around the world, accounting for 35–45% (around 3.7–4.8 million tons) of total worldwide annual edible mushrooms production (Masoumi et al. 2015). While using the spent mushroom substrate as a feedstuff in ruminant nutrition has largely been documented (Fazaeli and Masoodi 2006; Mahesh and Mohini 2013; Baek et al. 2017), information on the nutritive value and the possibility of using the bottom mushroom stipe in the diet of ruminants is scarce in the literature. Previous research has indicated that, in addition to bioactive compounds, button mushroom stipe has high carbohydrate (59.4–69.4%) and crude protein (16.8–29.4%) contents (OECD 2007; Nasiri et al. 2013; Baziuon et al. 2020). Hence, this by-product can be a good source of energy and protein for farm animals, which can replace part of fodder as well as protein concentrates in the diet of ruminants. We hypothesised that BMS with high carbohydrate and crude protein contents can replace part of the TMR diet of ruminants without negatively affecting their health and performance.

Therefore, the objectives of the present study were to determine the nutritive value and ruminal degradability of BMS using *in vitro* and *in sacco* methods, and evaluate its potential as a feedstuff for ruminants when included in a mixed ration for growing lambs.

**Materials and methods**

**Button mushrooms stipe and alfalfa samples**

The BMS samples were obtained from a commercial mushroom farm in Hamedan province. Several samples (10 samples of 200 kg, fresh weight basis) were taken from different parts of the cultivation rooms in the farm; the samples were then pooled to obtain a representative sample that was used for all three experiments. The BMS was firstly washed with tap water (because of contamination of stipes with soil and compost) and then air-dried at room temperature to a constant weight (around 81% DM content).

Alfalfa sample used in Exp.1 and Exp.3, was a representative sample prepared from the second cut alfalfa samples collected from three farms in Hamedan province.

**Experiments**

This study consisted of three experiments (Exp). In Exp.1, ruminal degradability characteristics of BMS were determined using the nylon bag technique. The second experiment (Exp.2) determined the gas production kinetics and ME content of BMS. The third experiment (Exp.3) was carried out *in vivo* to evaluate the impact of incorporating different levels of BMS into the ration of growing lambs on their feedlot performance, total tract digestibility, and blood metabolites.

**In sacco experiment**

Three ruminally fistulated Mehraban mature rams (50 ± 4.5 kg BW) were used for *in sacco* experiment. The rams were fed with a maintenance diet composed of (per kg DM) 300 g barley and 700 g alfalfa hay, providing 9.58 MJ ME and 137.4 g CP. For *in sacco* incubations, representative samples of BMS were ground to 2 mm, and sub-samples of 3 g (DM basis) were weighed into polyester bags (14 × 8 cm, pore size of 40–50 μm). The bags were heat-sealed and subsequently incubated for 2, 4, 8, 16, 24, 48, and 72 h in the rumen of rams. The bags were removed at the end of each incubation time and placed in ice water to stop the fermentation. The bags were then washed in a cool-rinse cycle by a washing machine for 20 min and subsequently dried at 55°C to a constant weight in a forced-air oven. Zero time washing losses were
determined by washing non-incubated bags in the same manner as described for incubated bags.

The incubations were repeated on two different days (six replications per sample for each incubation time). Data on ruminal DM and CP degradability were fitted to the following equation proposed by Orskov and McDonald (1979) using the NLIN procedure of SAS (SAS 2002).

\[ P = a + b(1 - e^{-c \times t}) \]

Where \( P \) is the DM or CP (% of initial amount) that disappeared at time \( t \), \( \alpha \) is the intercept representing the soluble fraction (% of DM or CP), \( \beta \) is the slowly degradable fraction (% of DM or CP), \( \gamma \) is the degradation rate of the fraction \( \beta \) (\%/h) and \( \tau \) is the time of incubation (h). The effective degradability (ED, %) of the samples was calculated using the following equation:

\[ ED = a + b \times \frac{c}{c + Kp} \]

Where \( 'kp' \) is the ruminal passage rate (\%/h).

**In vitro experiment**

*In vitro* gas production technique was used to estimate ME content (Gierus et al. 2008) and organic matter degradability (OMD) of BMS compared with those of alfalfa hay as standard hay (Menke and Steingass 1988). Briefly, the rumen fluid for *in vitro* incubations was collected before morning feeding from three ruminally fistulated rams fed the same diet as described for Exp.1. The rumen fluids were pooled and transferred into a pre-warmed insulated flask (with a temperature of about 38°C), and immediately transported to the laboratory.

For incubation of BMS and alfalfa hay, they were oven-dried at 55°C for 48 h and ground to pass a 1 mm sieve and sub-samples of 200 mg (DM basis) were incubated (six replicates per sample) with 30 ml of rumen inoculum in 100 mL glass syringes under CO2 atmosphere. In this experiment, the type of the fermentation substrate (alfalfa hay and BMS) and the syringes (six replicates for each substrate) were considered as treatment and the experimental units, respectively. For preparing the rumen inoculum, the rumen fluid was strained through four layers of cheesecloth and mixed with the buffer at a ratio of 1:2 (v/v) (Menke and Steingass 1988). Six syringes containing the buffered rumen inoculum without substrate were used as blanks. The syringes were incubated in a water bath at 39°C and the gas volume was recorded at 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, and 144 h of incubation. Alfalfa hay was also used as the standard hay to adjust the gas production data to its averaged data obtained from previous multiple incubations.

Data of gas production during 144 h of incubation were fitted to the model proposed by France et al. (2000) as shown below, by NLIN procedure of SAS (SAS 2002).

\[ GP = A \left\{ 1 - e^{-[b(t-1)+c(\sqrt{t}-1)]} \right\} \]

Where \( GP \) (ml) is the gas produced at the time \( t \), \( 'A' \) (ml) is the asymptote of gas production, \( 'b' \) and \( 'c' \) are constants and \( L \) (h) is the lag time.

Fractional gas production rate (\( \mu \)/h) was estimated at the time of half asymptotic gas production (\( T_{1/2} \) h), using the following equations as described by France et al. (2000):

\[ T_{1/2} = \frac{(-\frac{\pi}{2} + \sqrt{\left\{\frac{c^2}{4} + b(bL + c\sqrt{L} - \ln(0.5))\right\}}}{b} \]

\[ \mu = b + c/\left(2\sqrt{T_{1/2}}\right) \]

The ME, OMD, and DOMD of the samples were estimated based on the gas produced after 24 h of incubation using the following equations (Menke and Steingass 1988):

\[ ME = 1.06 + 0.157 \times GP + 0.0084 \times CP + 0.022 \times EE - 0.0081 \times Ash \]

\[ OMD = 14.88 + 0.889 \times GP + 0.45 \times CP + 0.651 \times Ash \]

\[ DOMD = OMD \times OM \]

Where, \( GP \) is the cumulative gas produced during 24 h of incubation (ml/200 mg DM of the substrate), \( EE \) is ether extract (g/kg DM), \( CP \) is crude protein content (g/kg of DM), \( ME \) is the metabolisable energy (MJ/kg DM), OMD (%) is organic matter degradability, and DOMD (%) of DM is the digestible organic matter content.

**In vivo experiment**

*Animals, experimental rations, and feeding procedure.* Animal feeding management in the current study was in agreement with an approved Bu-Ali Sina University Animal Care and Use Committee Protocol (Ref No: 1397/1139). In Exp. 3, 24 Mehraban male lambs, weighing 27.8 ± 3.90 kg, were stratified by weight and randomly assigned to one of the following dietary treatments: (1) the basal ration without BMS as the control (CTRL), (2) 10% (DM basis) replacement of the basal TMR ration by BMS (BMS10), and (3) 20% replacement of the basal TMR ration by BMS (BMS20). The
rations were formulated to obtain iso-energetic and iso-nitrogenous rations to meet the nutrient requirements of the lambs for an average daily gain (ADG) of around 250 g (NRC 2007). The ingredient and chemical composition of the rations are shown in Table 1. Lambs were housed in individual pens and received the experimental rations ad-libitum as total mixed ration twice daily at 08:00 and 17:00 h during 60 days of the growth performance period. Lambs also had free access to freshwater throughout the experiment. At the end of the performance period, lambs were transferred to individual metabolic crates and after an adaptation period of 14 days, faecal samples were collected for 6 days for determining the total tract digestibility of the nutrients in the experimental rations.

**Sampling procedures.** Feed offered and refusals were recorded daily before morning feeding to estimate daily feed intake. A representative sample of the feed (500 g) was distributed to each treatment group and that of refusals of each lamb (100 g) was taken once every two weeks during the performance period, and every day during the last week of the experiment. The samples were then dried at 55 °C in a forced-air oven, and those of digestibility period were ground to pass a 1-mm screen and stored at room temperature for subsequent analysis. The bodyweight of lambs was recorded once every two weeks before morning feeding to estimate ADG at two weeks intervals. Blood samples were collected from the jugular vein before morning feeding on days 30 and 60 of the performance period, centrifuged at 2000 × g to obtain serum samples, which were kept frozen for subsequent analysis. The rumen fluid samples were collected 3 h after morning feeding by stomach tubes on the last day of the performance period. Rumen fluids were then strained through four layers of cheesecloth, aliquots of 10 ml were acidified by an equal volume of 0.2 N chloridric acid and stored at −20 °C for determining the rumen ammonia concentration. An additional set of rumen fluid samples was fixed by orthophosphoric acid 25% (4:1, v/v) and stored at −20 °C for the rumen VFA content analysis.

**Chemical analyses**

The feed samples were pooled within treatment, and those of refusals and faeces were pooled by animal and analysed for dry matter (DM, ID no. 930.15), organic matter (OM, ID no. 920.05), ether extract (EE, ID no. 920.39), and crude protein (CP, ID no. 984.13) contents according to standard procedures of AOAC (AOAC 2000). The neutral detergent fibre was analysed using heat-stable alpha-amylase as described by Van Soest et al. (1991) and expressed exclusive of residual ash. Serum metabolites including, glucose, triglyceride, cholesterol, total protein, albumin, urea, creatinine, and serum enzymes including alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and glutathione peroxidase (GPx), were determined using commercially available kits (Pars Azmoon, Tehran). Ruminal ammonia concentration was determined calorimetrically according to the method elucidated by Broderick and Kang (1980). Ruminal total volatile fatty acids (TVFA) concentration was measured using a Markham apparatus as described by John et al. (1957). Briefly, a combination of 2 ml of the rumen inoculum, 1 ml 10% potassium oxalate buffer, and 1 ml oxalic acid was injected into the apparatus and a distillate of 100 ml was collected and subsequently titrated against a standard of 0.01 N NaOH using phenolphthalein as the indicator. TVFA concentration was then calculated using the following equation:

\[
TVFA \text{ (mM)} = \frac{\text{NaOH volume} \times \text{NaOH normality}}{\text{Rumen inoculum volume}} \times 1000
\]

**Statistical analysis**

Data on gas production kinetic parameters were analysed using the GLM procedure of SAS (2002) with the syringes as experimental units (six replications). Data on growth performance including daily dry matter intake (DMI), average daily gain (ADG), and feed

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**Table 1. Ingredient and chemical composition of the experimental rations and button mushroom stipe (BMS).**

| Items | CTRL | BMS10 | BMS20 | BMS | Alfalfa |
|-------|------|-------|-------|-----|---------|
| Alfalfa (g/kg, DM basis) | 302 | 167 | 150 | – | – |
| Wheat straw | 50 | 50 | 0 | – | – |
| Barley | 535 | 598 | 622 | – | – |
| Rapeseed meal | 103 | 75 | 17 | – | – |
| Button mushroom stipe | 0 | 100 | 200 | – | – |
| Salt | 5 | 5 | 5 | – | – |
| Vitamins-minerals premix | 5 | 5 | 5 | – | – |
| Diet cost (USD/kg) | 0.42 | 0.39 | 0.37 | – | – |

| Chemical composition (g/kg of DM) | | | | | |
| DM | 913 | 903 | 896 | 810 | 925 |
| OM | 906 | 892 | 891 | 812 | 949 |
| CP | 140 | 140 | 140 | 246 | 144 |
| aNDF | 382 | 357 | 343 | 294 | 484 |
| EE | 26 | 25 | 24 | 7 | 25 |
| NFC | 357 | 370 | 383 | 265 | 296 |
| ME (MJ/kg) | 11.30 | 11.30 | 11.30 | 7.70 | 9.70 |

CTRL: basal ration without BMS; BMS10: 10% BMS inclusion (DM basis) in the basal ration; BMS20: 20% BMS inclusion in the basal ration; DM: dry matter; OM: organic matter; CP: crude protein; aNDF: neutral detergent fibre (determined using heat stable alpha-amylase); ME: metabolisable energy; EE: ether extract; NFC: non-fibrous carbohydrates.
conversion ratio (FCR) estimated as kg DMI intake to kg daily weight gain ratio at the intervals of two weeks were analysed using the MIXED procedure of SAS (2002) with REML repeated measure and AR[1] covariance structure. The model included the fixed effects of treatment, day, and treatment x day, and random effect of animals nested within treatment, and initial bodyweight of the animals was considered as a covariate factor. Data on blood metabolites were analysed using the same model used for performance traits but without covariate factor. Data on digestibility were analysed using the GLM procedure. For the in vivo experiment, the animals were considered as experimental units (eight replications). Tukey’s multiple range test was used for the comparison of means and least-square-means, with a significant level declared at \( p < .05 \).

Results

In sacco ruminal degradation characteristics of BMS

Ruminal degradability kinetics of DM and CP of BMS are shown in Table 2. The ‘a’ and ‘b’ fractions in DM were comparable to those in CP of BMS, being around 40 and 60%, respectively; however, the degradation rate of ‘b’ fraction was around 30% higher in CP than in DM. Effective ruminal degradability of CP was also slightly higher than that of DM, being 81, 67, and 61% in the ruminal passage rates of 0.02, 0.05, and 0.08/h, respectively.

Gas production kinetics of BMS and alfalfa

Except for lag phase (L) that remained the same between alfalfa and BMS, all the other parameters of gas production kinetic were higher in alfalfa than in BMS (Table 3), as the asymptotic gas production, the gas produced after 24 h of incubation (GP24), OMD, DOMD and ME (as indices of fermentation extent) were 6.4, 21.3, 6.5, 14.7, and 20.6% lower, respectively, in BMS than in alfalfa (\( p < .05 \)). Alfalfa also fermented more rapidly compared with BMS (\( p < .05 \)), as its fractional gas production rate (\( \mu \)) was 46.5% higher than that of BMS.

Total tract digestibility and ruminal fermentation

Incorporating BMS into the ration of lambs had no effect on DM and OM digestibilities (\( p > .05 \)) (Table 4). Similarly, the CP and NDF digestibilities remained unaffected by treatment (\( p > .05 \)). The ruminal TVFA concentration was not changed by the treatments (\( p > .05 \)), however, the ruminal ammonia concentration decreased proportionally with including BMS in the ration of lambs (\( p < .05 \)), as the lowest ruminal ammonia was observed in lambs fed BMS20.

| Table 2. In sacco ruminal degradation kinetics of dry matter (DM) and protein (CP) of button mushroom stipe (BMS). |
|-----------|-------------|-------------|---------|
| Parameters | DM          | CP          |
| \(\alpha\) (%) | 42.1 ± 1.36 | 43.3 ± 1.38 |
| \(b\) (%)    | 57.0 ± 1.38 | 59.1 ± 1.23 |
| \(c\) (1/h)  | 0.026 ± 0.002 | 0.034 ± 0.001 |
| ED (%)       | 0.02         | 74.9 ± 0.09 |
|             | 0.05         | 62.2 ± 0.28 |
|             | 0.08         | 56.7 ± 0.53 |
| \(L\) (h)    | 0.0025       | 0.005       |
| \(m\) (h)    | 0.046b       | 0.086a      |
| GP24 (mL)    | 38.80b       | 49.30a      |
| OMD (%)      | 61.60        | 65.90       |
| DOMD (%)     | 50.10b       | 58.70a      |
| ME (MJ/kg DM)| 7.70b        | 9.70a       |

\(a, b\): Different letters in the same row indicates significant differences (\( p < .05 \)). Different superscript letters indicate differences (\( p < .05 \)) between the groups.

| Table 3. Gas production kinetic parameters of button mushroom stipe (BMS) and alfalfa. |
|--------|---------|---------|---------|
| Feeds  | BMS     | Alfalfa | SEM     | p-Value |
| \(A\) (mL) | 52.70b  | 56.30a  | 0.72    | .012    |
| \(L\) (h)  | 0.0025  | 0.005   | 0.0027  | .537    |
| \(\mu\) (1/h) | 0.046b  | 0.086a  | 0.0016  | <.001   |
| GP24 (mL) | 38.80b  | 49.30a  | 0.35    | <.001   |
| OMD (%)   | 61.60   | 65.90   | 0.30    | <.001   |
| DOMD (%)  | 50.10b  | 58.70a  | 0.27    | <.001   |
| ME (MJ/kg DM)| 7.70b  | 9.70a   | 0.05    | <.001   |

\(A\): asymptotic gas production (per 200 mg DM); \(L\): lag time; \(\mu\): fractional gas production rate; GP24: the gas produced over 24 h of incubation; OMD: organic matter digestibility; DOMD: digestible organic matter content; ME: metabolisable energy; SEM: standard error of the means.

| Table 4. In vivo total tract nutrient digestibility and ruminal fermentation of the experimental rations. |
|--------|---------|---------|---------|---------|
| Experimental rations | CTRL | BMS10 | BMS20 | SEM     | p-Value |
| DM (%)   | 71.5   | 75.5   | 72.5   | 1.73    | .291    |
| OM (%)   | 74.1   | 77.8   | 74.7   | 1.59    | .273    |
| NDF (%)  | 43.8   | 45.7   | 46.9   | 7.23    | .953    |
| CP (%)   | 75.7   | 73.3   | 72.4   | 1.79    | .432    |
| TVFA (mmol/L)| 62.8  | 69.1   | 68.5   | 4.95    | 1.999   |
| NH\(_3\)-N (mg/dL)| 24.7a | 22.7a  | 17.9a  | 1.08    | <.001   |

| Nutrients | (\% of DMI) | CP | DM | OM | NDF | TVFA | NH\(_3\)-N |
|-----------|-------------|----|----|----|-----|------|-----------|
| CTRL      | 71.5        | 43.8| 74.1| 43.8| 62.8| 24.7a|
| BMS10     | 75.5        | 45.7| 77.8| 45.7| 69.1| 22.7a|
| BMS20     | 72.5        | 46.9| 74.7| 46.9| 68.5| 17.9a|

Nutrient digestibility (%): DM: dry matter; OM: organic matter; NDF: neutral detergent fibre; CP: crude protein; TVFA: total VFA concentration; CTRL: basal ration without BMS; BMS10: 10% BMS inclusion (DM basis) in the basal ration; BMS20: 20% BMS inclusion in the basal ration; SEM: standard error of the means. Different letters in the same row indicate significant differences (\( p < .05 \)).

Growth performance

Including BMS in the ration of growing lambs did not affect their growth performance (\( p > .05 \)) (Table 5). The body weight remained the same among the
tended to consume less feed than the lambs ration (experimental period), DMI was not affected by treatment during the whole experimental period, those fed CTRL ration. exhibited an 11.3 and 14.8% more growth rate than lambs consuming both BMS 10 and BMS 10 rations, significant effect on their ADG and FCR, however, weeks of the experiment (d15 1078 1069 1051 12.20 .955 .067 0.087 d30 31.90 32.80 32.40 1.31 .902 .05 .773 d60 40.40 41.10 41.70 1.77 .887 .001 .026 Table 5. Feedlot performance of lambs fed the experimental rations.

BW: body weight; DMI: daily dry matter intake (g per animal per day); ADG: average daily gain; FCR: average feed conversion ratio (g daily dry matter intake/g daily weigh gain); CTRL: basal ration without BMS; BMS10: 10% BMS inclusion (DM basis) in the basal ration; BMS20: 20% BMS inclusion in the basal ration; SEM: standard error of the means; Tr: treatment.

| Items          | CTRL   | BMS10 | BMS20 | SEM  | Tr | Day | Tr × Day |
|----------------|--------|-------|-------|------|----|-----|----------|
| BW (kg)        | 33.70  | 34.70 | 35.00 | 0.61 | .279 | .067 | .087     |
| d0             | 1498   | 1488  | 1469  | 13.50 | .34 | .203 | .026     |
| d30            | 1078   | 1069  | 1051  | 12.20 | .326 | –    | –        |
| d60            | 1526   | 1525  | 1507  | 5.80  | .060 | –    | –        |
| d15–30         | 1641   | 1641  | 1676  | 20.80 | .280 | –    | –        |
| d30–60         | 1758   | 1721  | 1683  | 31.70 | .201 | –    | –        |
| ADG (g/d)      | 230    | 256   | 264   | 15.10 | .276 | .043 | .313     |
| FCR            | 6.66   | 5.83  | 5.70  | 0.35  | .139 | .013 | .773     |

The DMI was not affected by treatment during the whole experimental period (p > .05); however, lambs receiving BMS20 tended to consume less feed than the lambs in the other groups during the second two weeks of the experiment (p = .06).

Including BMS in the ration of the lambs had no significant effect on their ADG and FCR, however, lambs consuming both BMS10 and BMS10 rations exhibited an 11.3 and 14.8% more growth rate than those fed CTRL ration.

**Blood metabolites**

None of the serum metabolites (Table 6) was affected by treatment, however, total protein tended to increase in the lambs fed BMS10 (p = .089).

Both ALT and AST were not affected by treatment (p > .05), however, ALP decreased with the inclusion of BMS in lambs ration (p = .030), as the lambs fed BMS10 had a lower ALP than those fed CTRL during the whole and the first half of the experimental period (p < .05). Blood creatinine and GPx did not differ among the treatments throughout the experiment (p > .05).

**Discussion**

The principal objective of this study was to determine the nutritional value of BMS as a new cheap by-product feedstuff for ruminants. Hence, the first experiment (Exp.1) was conducted to evaluate the ruminal degradability characteristics of the BMS crude protein, and the second experiment (Exp.2) was performed to determine its energetic value. The information from the first two experiments was used to formulate the experimental rations in the last experiment. In Exp.3, BMS replaced part of the conventional feedstuffs in a practical growing diet to investigate its impact on lambs health and performance.

**In sacco ruminal degradability**

In sacco results indicate that a major part of BMS crude protein has a slow degradability. As stated earlier, there is no comparable data on ruminal degradability of BMS in the literature; however, in a recent experiment conducted in vitro, Baziuon et al. (2020) determined the ruminal degradability characteristics of white button mushroom stem (WBMS) compared with alfalfa hay. The WBMS used in that experiment was a by-product comparable to BMS in the current study but with a lower CP, ash, and NDF; and a higher EE content than those of BMS (16.8, 8.6, 21.5, and 3.3% vs. 24.6, 18.8, 29.4, and 0.7% for CP, ash, NDF, and EE, respectively). In that experiment, the ‘d’ , ‘b’, and the ‘b’ degradation rate of WBMS dry matter were 39.7, 53.5%, and 0.03 /h, respectively. The first two values are fairly lower than those obtained in the current in sacco experiment. This difference might be related to different chemical compositions (especially a higher EE content in WBMS), and different experimental conditions that typically exist between in vitro and in sacco experiments. In Baziuon et al. experiment, the WBMS had higher ‘d’ and ‘b’ fractions, and a lower ‘b’ degradation rate than alfalfa hay (39.7, 53.5%, and 0.03 vs. 24.5%, 26.9%, and 0.065, respectively).
However, the current in sacco results was in line with those of earlier studies, indicating that a small fraction of N (around 30–40%) in button mushroom stipe is soluble (OECD 2007; Cherno et al. 2013).

In the current study and the third experiment, BMS replaced mainly alfalfa, rapeseed meal, and wheat straw in the growing lambs ration. However, it is difficult to have a reliable comparison between CP fractions in BMS and those in the above-mentioned feedstuffs because of a wide range of data reported for CP fractions of these feedstuffs in the literature. In total, regarding the ruminal degradation characteristics of alfalfa CP in the literature (the ‘α’, ‘b’, and ‘c’ parameters of around 22–57, 35–53%, and 0.067–0.197, respectively) (NRC 2001; Yu et al. 2004; Valderrama and Anrique 2011; AFZ 2011; Coblentz and Grabber 2013; Aghajanzadeh-Golshani et al. 2015) and those of rapeseed meal (the ‘a’, ‘b’, and ‘c’ parameters of around 3–31, 61–92%, and 0.068–0.145, respectively) (NRC 2001; Wulf and Südekum 2005; AFZ 2011; Steingass et al. 2013; Nedelkov et al. 2017), and wheat straw (the ‘a’, ‘b’, and ‘c’ parameters of around 38, 26%, and 0.056, respectively) (AFZ 2011), it can be concluded that BMS has a slower ruminal degradation than all the feedstuffs it replaced in the diet.

**Gas production kinetics**

In Exp.2, the asymptotic gas production, as an index of fermentation extent in long-term incubation, was lower in BMS than in alfalfa, implying that BMS has a lower energetic value compared with alfalfa.

These results were fairly inconsistent with those obtained by Baziuon et al. (2020), who reported a higher energetic value for WBMS than alfalfa hay (OMD and ME values of 59.6% and 8.01 MJ/kg, respectively, for WBMS vs. 53.9% and 7.15 MJ/kg, of alfalfa hay). These contradictions, as stated earlier, are most probably related to the difference in the chemical composition of the substrates used in these experiments.

In the current study, regarding the chemical composition of BMS and alfalfa hay, the higher ash content in BMS than in alfalfa seems to be the major cause of lower asymptotic gas production, and by consequence, the lower energetic value estimated for BMS compared with alfalfa hay. Because in long-term incubation, BMS had an even higher asymptotic gas production than alfalfa (129.8 vs. 124.6 ml) based on 1 g OM incubated. Moreover, it has been well-known that proteins have a lower contribution than carbohydrates in gas production, resulting in a low amount of gas produced in the feedstuffs with high protein content (Makkar 2004). Therefore, a higher CP content in BMS is another major cause, lowering the asymptotic gas production as well the energetic value of BMS compared with alfalfa hay. However, a slower ruminal degradability and fermentation of BMS, as confirmed by lower gas production and CP degradation rates, have also contributed to the BMS lower ME compared with alfalfa. This is supported by the fact that the difference between alfalfa and BMS in terms of their GP24 and other parameters estimated based on GP24, including OMD, DOMD, and ME, were more pronounced than that of asymptotic gas production estimated based on the gas produced over 144 h of incubation.

Indeed, the composition of fermentable carbohydrates is one of the key factors determining the degradation and fermentation rate of substrates in the rumen (Makkar 2004). Readily fermentable carbohydrates, with typically higher degradation and fermentation rates than cell walls, have been reported to have a major contribution to gas production by providing immediate energy to rumen microorganisms that may enhance cell walls degradation (Salama et al. 2020). In the current study, NFC, an index of readily fermentable carbohydrates, was lower in BMS than in alfalfa, causing a slower ruminal degradability and a lower gas production rate of BMS compared with alfalfa. Hence, a lower NFC accompanied by a higher CP probably caused a slower ruminal degradability and fermentation of BMS, resulting in a lower ME obtained for BMS compared with alfalfa hay.

**In vivo digestibility and fermentation**

In the present study, the BMS energy and CP contents were not comparable to those of any of the feedstuffs used in the ration of growing lambs, therefore, BMS substituted partially for alfalfa, rapeseed meal, and wheat straw (in BMS20) to obtain iso-energetic and iso-nitrogenous rations in all the experimental groups. Although obtaining the rations with identical chemical composition in this type of experiment is not possible, however, BMS replaced the basal ration ingredients in such a way that the chemical composition of the rations to be comparable, though NDF content of BMS10 and BMS20 was slightly lower than the control. Partial replacement of these conventional feedstuffs with BMS (with a relatively slower ruminal degradability and fermentation than the feedstuffs it replaced) in lambs diet, had no negative impact on the nutrient total tract digestibility and ruminal TVFA
concentration. Mathematically, although the negative effect of BMS on the whole ration digestibility could be negligible, however; BMS inclusion in lambs rations numerically improved nutrients total digestibility, and these results were proportionally consistent with a relatively high TVFA and an improved growth performance of lambs in BMS10 and BMS20. This might be partly due to a balanced and synchronised supply of nutrients from BMS in combination with those from other feedstuffs to rumen microorganisms improving, thus the efficiency of microbial protein synthesis. In this context, Soltan et al. (2021) reported that partial replacement of maize with sorghum grain (with a typically lower digestibility than maize) in the diet of lambs tended to improve ruminal microbial protein synthesis. Research has indicated that white button mushroom is a good source of protein (19–43%, DM weight basis), carbohydrate (43–61%), and minerals (OECD 2007; Cherno et al. 2013; Usman et al. 2021). The white button mushroom contains both non-protein nitrogen (around 36–38% of total N) and true protein supplying all the essential amino acids (accounting for 32–43% of its total amino acids content) (OECD 2007; Cherno et al. 2013). Nearly 50% of the button mushroom amino acids are in free form that is considered as a valuable readily available N for rumen microorganisms (OECD 2007). Moreover, the mono-, di- and oligosaccharides (mainly including glucose and trehalose), and mannitol, as the soluble sugars, accompanied by glycogen (making together more than half of the button mushroom carbohydrates content) are the readily fermentable carbohydrates (Beelman et al. 2003; OECD 2007; Usman et al. 2021) that can be rapidly fermented and provide immediate energy to rumen microorganisms. However, the dominant polysaccharides in the white button mushroom cell wall, including chitin, β-glucans, and hemicellulose, are also fermented at a lower rate by rumen microorganisms (Fadel El-Seed et al. 2003; Miltko et al. 2010). White button mushroom is also rich in minerals (mainly including, potassium, phosphorus, sodium, calcium, and magnesium; and trace elements like copper, zinc, and iron) which can fulfill the requirements of rumen microorganisms to minerals (Beelman et al. 2003; Cherno et al. 2013; Usman et al. 2021).

In addition, previous in vitro experiments have revealed that certain biologically active substances in medicinal mushrooms may affect the rumen microbial ecosystem by altering the rumen dominant microbial populations in a dose-dependent manner. In this regard, Yeo et al. (2011) and Kim et al. (2014) reported that the mycelia of cordyceps fungus enhanced ruminal digestibility of the substrate and improved rumen fermentation by increasing VFA production and abating methanogenesis at doses up to 0.25 g/L. In a recent experiment, Chanjula and Cherdthong (2018) found that supplementing goats diet with cordyceps spent mushroom at 100 g/d intake level, improved DM, OM, and NDF apparent digestibility in the diet. In another experiment, treating corn field residue and rice straw with white-rot fungi (Pleurotus ostreatus and Volvariella volvacea) improved their ruminal digestibility and fermentation (Khonkhaeng and Cherdthong 2020). There is also a wide range of bioactive compounds in button mushrooms, which could positively modulate rumen fermentation and improve the gut function in ruminants. In this connection, phenolic compounds, terpenoids, chitosan, and ribotoxins extracted from white button mushrooms have been found to exert antimicrobial activity (Muszyńska et al. 2017; Rezaeian and Pourianfar 2018; Usman et al. 2021), thus these substances have the potential to affect rumen microbial ecosystem by inhibiting mainly gram-positive bacteria (Dhamodharan and Mirunalini 2010). There are also data reporting antimicrobial activity from white button mushroom aqueous extract against pathogenic bacteria like Staphylococcus aureus and Escherichia coli (Tehrani et al. 2012; Risan et al. 2017), which could improve the gut health and function (Fard et al. 2014). The antioxidant property of phenolic compounds in BMS might also have a positive effect on the rumen microbial protein synthesis. This is supported by previous research that found that natural antioxidants like polyphenols enhance the amount and efficiency of the rumen microbial protein synthesis (Cattani et al. 2012; Zeoula et al. 2019). Furthermore, the oligo- and polysaccharides present in white button mushrooms have been found to possess prebiotic activity (Aida et al. 2009) and could positively modify rumen fermentation. This is supported by the findings of Tian et al. (2018), who reported that feeding mice with a diet supplemented with white button mushroom altered the gut microbiota, favouring the bacteria producing mainly propionate.

A proportionally decreased concentration of ruminal ammonia with BMS inclusion in the ration might be linked to the low ruminal degradability of crude protein in BMS. In general, the ammonia concentration in the rumen is affected by ruminal protein degradation on one hand and ammonia uptake by the rumen bacteria on the other hand (Nolan and Dobos 2005). Hence, the low ruminal ammonia concentration in the BMS-containing rations might also be a result of higher microbial protein synthesis. In total, regarding
no change in CP total tract digestibility, a decreased ammonia concentration could be considered a beneficial effect of BMS inclusion in the ration of lambs.

**Growth performance**

Although the inclusion of BMS in the ration of lambs had no significant effect on their growth performance, however, the average daily gain increased numerically by 11.7 and 14.8% in the lambs receiving BMS$_{10}$ and BMS$_{20}$ respectively, compared with CTRL. Moreover, including BMS in the ration decreased diet costs by 7.2 and 14.5% in BMS$_{10}$ and BMS$_{20}$ respectively. This could probably be a result of, as mentioned previously, a slightly improved rumen fermentation and nutrient total tract digestibility in lambs fed BMS-containing rations. In this regard, previous research has indicated that white button mushroom contains propionate, a key energy source for ruminants, which induces insulin secretion in the body (Usman et al. 2021). As stated earlier, white button mushrooms can also promote propionate production in monogastric, thus, it seems to have the potential to modify rumen fermentation towards producing more propionate, which could improve the feed energy utilisation in ruminants (Aida et al. 2009; Tian et al. 2018). However, these effects remained to be explored in vitro and in vivo. Moreover, antioxidant and anti-inflammatory properties reported for the button mushroom certain bioactive compounds, like phenolic compounds, flavonoids, and fibres (Usman et al. 2021), might also have a contribution to the slightly improved growth performance of growing lambs receiving BMS$_{10}$ and BMS$_{20}$. There is no information on the use of mushrooms in the diet of ruminants in the literature; however, some previous research has shown a positive impact of mushrooms on broiler’s performance when included in their diet (Guo 2003; Fard et al. 2014). The improvement of broiler performance in those studies was linked to different factors including suppressing pathogenic and promoting commensal bacteria in the gut, boosting the immune system, and increasing selenoenzymes activity due to the high antioxidant activity of mushrooms (Giannenas et al. 2010; Fard et al. 2014; Lee et al. 2015). Regarding a relatively high nutritional value of BMS ($CP = 24.6\%$ and $ME = 7.8 \text{MJ/kg}$) and the fact that BMS had no adverse effect on growing lambs performance, it can be concluded that including BMS in ruminant diet can be cost-effective.

**Blood metabolites**

All the blood metabolites, including nutritional status indices, hepatic enzymes, renal function indices, and antioxidant capacity enzyme, were within the reference optimal ranges (Radostitis et al. 2007).

One great part of the health-promoting effects of mushrooms observed mainly in unhealthy and experimentally infected animals has been attributed to mushrooms bioactive compounds. Hence, for preserving these bioactive substances, mushrooms by-products need to be used freshly or in freeze-dried form to be effective (Guo 2003; Giannenas et al. 2010; Stojković et al. 2014; Rathore et al. 2017). In ruminants, most of these bioactive compounds may be degraded or become inactive in the rumen because of its high proteolytic activity, which needs to be investigated in vitro and in vivo. The decreased ALP concentration, as an index of liver health, in lambs fed BMS might be related to the mushroom anti-inflammatory activity. In this context, research has indicated that white button mushrooms are rich in zinc, which exerts strong anti-inflammatory activity (Prasad 2014). Moreover, button mushrooms are also a natural reservoir of ergosterols, characterised by exhibiting a potent anti-inflammatory activity (Elsayed et al. 2014).

**Conclusions**

The results from this study revealed that BMS has a relatively high crude protein content (comparable to beans) but with a relatively low ruminal degradation rate that could be a good source of protein for ruminants. The gas production kinetic parameters indicated that BMS has a slow ruminal degradability and fermentation because of its high ash and crude protein content, negatively influencing its energetic value. Partial replacement of conventional feedstuffs with BMS in the diet of growing lambs reduced diet cost without adversely affecting their health and growth performance. Additionally, including BMS in the diet had no adverse effect on feed intake and nutrient digestibility but reduced ruminal ammonia concentration that is of nutritional as well as environmental importance. In total, these findings suggest that BMS can be considered a valuable by-product feedstuff, which could be included in the diet ruminants at a level up to 20%.

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Ethical approval
All the experimental procedures and animal care protocols were approved by the Bu-Ali Sina University Animal Care and Use Committee.

Disclosure statement
The authors declare that there is no conflict of interest.

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Data availability statement
The data analysed during the current study are available from the corresponding author on reasonable request.

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