

Different milling byproduct supplementations in mushroom production compost composed of wheat or rice straw could upgrade the properties of spent mushroom substrate as a feedstuff

Diferentes suplementações de subprodutos da moagem no composto de produção de cogumelos composto de palha de trigo ou arroz poderiam melhorar as propriedades do substrato de cogumelo usado como alimento

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ABSTRACT

Wheat bran (WB), barley flour (BF), rice bran (RB), wheat red dog (WRD) or reduction shorts (WRS) supplementation, as a food supplement (FS, 19%), to mushroom composts having wheat (WS) or rice straw (RS) as basal substrate (BS, 80%) could have contributed to improving the nutritional value and forage quality properties of spent mushroom substrate (SMS). The SMSs from king oyster mushroom (*Pleurotus eryngii*) production were evaluated with 2 BS (WS and PS) × 5 FS (WB, BF, RB, WRD, or WRS) factorial arrangement design. The BS × FS interaction significantly affected some of the studied variables (nutrient contents and *in vitro* true digestibility, relative feed value, and relative forage quality) of the SMSs used to be evaluated as a feedstuff. The BS significantly affected the acid detergent lignin content, digestible dry matter, metabolizable energy, estimated net energy, and total digestible nutrients. In contrast, the FS affected the acid detergent lignin content and net energy lactation value. In conclusion, independent of FS, WS-based SMSs and the BF and WRD supplemented-SMSs independent of BS had higher nutritional value and forage quality properties than other SMSs. Therefore, these SMSs could be exploited post-cultivation as animal feed due to their upgraded properties.

Index terms: Agricultural wastes; mushroom byproducts; mushroom compost; forage; feed-nutritive value.

RESUMO

Suplementação com farelo de trigo (WB), farinha de cevada (BF), farelo de arroz (RB), trigo red dog (WRD) ou redução short (WRS), como suplemento alimentar (FS, 19%), para compostos de cogumelos contendo trigo (WS) ou palha de arroz (RS) como substrato basal (BS, 80%) poderiam ter contribuído para melhorar o valor nutricional e as propriedades de qualidade da forragem do substrato de cogumelo gasto (SMS). Os SMS da produção de cogumelo ostra (*Pleurotus eryngii*) foram avaliados com planejamento fatorial 2 BS (WS e PS) × 5 FS (WB, BF, RB, WRD ou WRS). A interação BS × FS afetou significativamente algumas das variáveis estudadas (conteúdo de nutrientes e digestibilidade verdadeira *in vitro*, valor alimentar relativo e qualidade relativa da forragem) dos SMS utilizados para serem avaliados como alimento. A BS afetou significativamente o teor de lignina detergente ácido, matéria seca digestível, energia metabolizável, energia líquida estimada e nutrientes digestíveis totais. Em contraste, o FS afetou o teor de lignina em detergente ácido e o valor da energia líquida de lactação. Em conclusão, independentemente do FS, os SMS baseados em WS e os SMS suplementados com BF e WRD independentes do BS apresentaram maior valor nutricional e propriedades de qualidade da forragem do que outros SMSs. Portanto, estes SMSs poderiam ser explorados pós-cultivo como ração animal devido às suas propriedades melhoradas.

Termos para indexação: Resíduos agrícolas; subprodutos de cogumelos; composto de cogumelos; forragem; valor nutritivo da ração.

Introduction

Many agricultural enterprises, including mushroom production facilities, must overcome the significant challenges and costs (Singh et al., 2017; Gao et al., 2023) associated with reducing or even eliminating the production of useless waste. Indeed, edible mushroom production results in the release of large amounts of waste (30-60 million tons per year) or a material (5-6 kg of SMS per kg of edible mushroom) called spent mushroom substrate (SMS), which must be eliminated (Atallah et al., 2021). The SMS, an organic byproduct of the mushroom industry rich in nutrients (Rasib et al., 2015), generally comprises abiotic substances and biotic components such as fungal mycelia, bacterial biomass, and extracellular enzymes (Leong et al., 2022). Also, the SMS contains significant amounts of natural bioactive components, such as chitinous biopolymers, polyphenols, and melanin, with unique biological properties (Antunes et al., 2020). The SMS's

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moisture and fiber contents are relatively high, while relatively low in protein content (Hanafi et al., 2018).

The most effective way to overcome the difficulties in removing or storing the SMS is to use it as a soil conditioner or primarily as a feed ingredient (Sardar et al., 2017; Hanafi et al., 2018). The nutrient contents and biological properties of SMS as a feedstuff vary depending on the species of mushroom produced and the compost materials (Oh et al., 2010; Leong et al., 2022). However, the SMS from king oyster mushroom (*Pleurotus eryngii*) and oyster mushroom (*Pleurotus ostreatus*) production had a similar effect on rumen parameters such as pH and the concentrations of ammonia-N and volatile fatty acids (Oh et al., 2010). Also, agro-based SMS, which has a lower content of neutral detergent fiber (NDF) and better ruminal degradability, has the potential as an additional supplement for ruminant animals (Hanafi et al., 2018; Leong et al., 2022; Gao et al., 2023).

Straw management options, such as straw-based mushroom and feed production, are critical in sustainable straw use, including rice straw (RS). However, because of its chemical and physical structure, which is mainly composed of lignin, cellulose, hemicellulose, and silica, RS is resistant to bacterial degradation (Gu et al., 2014) and subsequently unsuitable for ruminant nutrition (Hanafi et al., 2018; Madzingira et al., 2021). Composting of the straws for edible mushroom production is an aerobic, solid-phase, self-heating process driven by the microbiological decomposition of organic materials (Souza et al., 2016; Wang et al., 2016). During this process, extracellular enzyme complexes (cellulase, cellobiase, hemicellulase, and lignin-modifying enzymes such as laccase, lignin peroxidase, manganese peroxidase, and versatile peroxidase) of mushrooms enhance the degradation of composts consisting of straws and ultimately positively affect the nutrient composition and nutritive value of the SMS (Kim et al., 2012; Sardar et al., 2017). Indeed, biological pretreatment using microorganisms such as edible fungi has facilitated lignocellulose conversion into sugars or other products without harsh chemicals and high temperatures (Wu et al., 2021), depending on the mushroom mycelia contained (Antunes et al., 2020). Therefore, RS is a suitable material for sustaining mushroom growth as well as conventional wheat straw (WS) compost (Wang et al., 2016).

King oyster mushroom, one of the most cultivated mushroom species in the world, requires compost materials rich in polysaccharides and nitrogen for its growth and development (Jeznabadi et al., 2017). Supplements from organic sources compensate for the probable nutrient deficiencies of the mushroom composts and thus enhance the growth of mushrooms (Jeznabadi et al., 2017; Dayani et al., 2018). Accordingly, the production of king oyster mushrooms generally takes place on WS-based substrate combined with food supplements (FS), such as milling byproducts, to overcome nutritional deficiencies (Jeznabadi et al., 2017; Kumar et al., 2021). Indeed, using WS as basal substrate (BS) and wheat (WB) or rice bran (RB) as FS is more common than other lignocellulosic (wood sawdust,

corn stalks and cobs, cotton waste, corn cobs, and grain straws) materials (Kim et al., 2012; Moradzadeh-Somarin et al., 2021). From an economic point of view, the biomass production (from 108.5 to 454.4 mg g dry weight⁻¹) and biological efficiency (85.2 to 146.3%) obtained on these BSs (Kirbag & Akyuz, 2008; De Pretto et al., 2018; Melanouri, Dedousi, & Diamantopoulou, 2022) for oyster mushrooms (*P. ostreatus* and *P. eryngii*) are over the values suggested for these variables (Sardar et al., 2016).

The milling byproducts such as WB, barley flour (BF), rice bran (RB), wheat red dog (WRD), or reduction shorts (WRS), which differ in chemical composition and biochemical and technological properties (Kaprelyants, Fedosov, & Zhygunov, 2013), as the FS source are not only added to provide adsorbent properties but also serve as a source of macro- and micro-nutrients (Jeznabadi et al., 2017; Hanafi et al., 2018). Accordingly, these FSs can improve the roughage quality parameters such as metabolizable energy (ME), relative feed value (RVF), and relative forage quality (RFQ) of the obtained SMS. However, information on whether the SMS's nutritional composition and feed value can be affected by different BSs and FSs that comprise the compost is limited. Moreover, the effects of the different BSs (WS vs. RS) with FSs (WB vs. BF, RB, WRD, or WRS) on the SMS's nutritive value and forage quality parameters from king oyster mushroom production are rarely compared. Therefore, this study evaluated the nutritional value and forage quality parameters of SMSs from WS- or RS-based mushroom composts supplemented with WB, BF, RB, WRD, or WRS as FS.

Material and Methods

Experimental design

The SMSs used in the study were obtained from a factorial experiment (2 BS sources × 5 FS sources) with three replicates in a completely randomized design that was conducted to evaluate the effects of different BSs (WS vs. RS) and FSs (WB, BF, RB, WRD or WRS) on the production efficiency of king oyster mushroom. The pre-cultivating chemical composition of mushroom substrates consisting of 80% BS, 19% FS, and 1% marble powder were calculated as presented in Table 1. In addition, the productivity and biological efficiency of the mushrooms that generated the spent mushroom substrates are presented in Table 1.

Approximately 5 kg of fresh SMS was collected from each treatment's three replicates and labeled on the day of the mushroom harvest. The SMS samples were immediately transferred to the laboratory (Department of Animal Science, Faculty of Agriculture, Ondokuz Mayıs University), where analyses were performed. The natural DM content of SMS samples was determined by drying to constant weight at 65°C for 48 hours (Association of Official Agricultural Chemists - AOAC, 2005). Thus, all samples brought to an air-dry basis (30 samples) were ground to pass through a 1 mm sieve using a sample mill and stored until analysis.

Table 1: The pH values and chemical properties (%) of the composts (basal substrate of 80% + food supplement of 19% + 1% marble power) used in the production of king oyster mushrooms (*Pleurotus eryngii*) and their mushroom yield (g kg compost⁻¹) and biological efficiency (%).

Compost	pH	Moisture	OM	Ash	C	CP	C/N	MP	BE
WS+BF	6.00	67.60	95.12	4.88	47.56	3.75	97.27	215.5	52.9
WS+WRD	5.92	69.93	95.00	5.19	47.50	5.00	59.38	238.0	65.4
WS+WB	6.51	72.85	95.41	5.43	47.71	4.56	65.36	104.9	28.8
WS+RB	5.82	71.11	94.96	6.04	47.48	5.06	58.62	233.3	61.4
WS+WRS	5.75	68.24	95.47	4.53	47.74	4.75	62.82	236.2	64.9
RS+BF	5.25	74.07	87.78	12.22	43.89	4.44	61.82	199.8	54.9
RS+WRD	5.00	69.65	87.47	12.53	43.74	5.63	48.60	145.6	40.0
RS+WB	5.22	71.46	87.23	12.77	43.62	5.19	52.55	204.9	56.3
RS+RB	5.54	68.54	86.62	13.38	43.31	5.69	47.59	193.0	53.0
RS+WRS	5.67	69.64	88.13	11.87	44.07	5.31	51.85	146.8	40.3

WS, wheat straw; RS, rice straw; WB, wheat bran; BF, barley flour; RB, rice bran; WRD, wheat red dog; WRS, wheat reduction shorts; OM, organic matters; C, carbon; CP, crude protein; C/N, carbon to nitrogen ratio; MY, mushroom yield; BE, biological efficiency.

Proximate analysis

The DM (method 930.15), ash (method 942.05), crude protein (CP, method 976.05), ether extract (EE, method 920.39), and crude cellulose (CC, method 930.10) analyses of SMS samples were performed using approved methods (AOAC, 2005). Nitrogen (N) content was determined using the Kjeldahl method (Büchi Distillation unit K-350), and a coefficient of 6.25 ($N \times 6.25$) was used to estimate the CP content. The EE content was determined using an automatic fat extraction (ANKOMXT15 Extractor) system, as Seenger et al. (2008) described. The CC content was determined using a Weende analysis (Carrier et al., 2011). The NDF and ADF contents were determined following the literature (Van Soest, Robertson, & Lewis, 1991) using the ANKOM A200/220 Fiber Analyzer (ANKOM Technology Corp., Fairport, NY, USA). The OM and nitrogen-free extracts (NFE) contents and carbohydrate fractions such as cellulose (CEL), hemicellulose (HC), and non-fibrous carbohydrate (NFC) of the samples were calculated using Equation 1, 2, 3, 4, and 5, respectively (Wahyono, Indiramata, & Human, 2021). All analysis results are expressed on a DM basis (% of DM).

$$OM, \% = DM - Ash \quad (1)$$

$$NFE, \% = OM - (CP + EE + CC) \quad (2)$$

$$CEL, \% = ADF - ADL \quad (3)$$

$$HCEL, \% = NDF - ADF \quad (4)$$

$$NFC, \% = (CP + (NDF \times 0.93) + EE + Ash) \quad (5)$$

Quality indicators and energy value

The digestible dry matter (DDM, Equation 6), dry matter intake (DMI, Equation 7), RFV (Equation 8), and RFQ (Equation 9) were determined using the index recommended by Undersander, Moore and Schneider (2010).

$$DDM, \% \text{ of } DM = 88.9 - (0.799 \times ADF) \quad (6)$$

$$DMI, \% \text{ of body weight} = 120 / NDF \quad (7)$$

$$RFV = (DMI, \text{ as } \% \text{ of body weight} \times DDM, \text{ as } \% \text{ of } DM) / 1.29 \quad (8)$$

$$RFQ = (ADF, \text{ as } \% \text{ of } DM \times TDN, \%) \quad (9)$$

The total digestible nutrients (TDN), ME, net energy lactation (NEL), and estimated net energy (ENE) values were calculated using Equation 10, 11, 12, and 13 (Pflueger et al., 2020; Wahyono, Indiramata, & Human, 2021).

$$TDN, \% = 4.898 + (89.796 \times (1.0876 - (0.0127 \times ADF, \text{ as } \% \text{ of } DM))) \quad (10)$$

$$ME, MJ / \text{ kg } DM = (0.17 \times DDM, \text{ as } \% \text{ of } DM) \quad (11)$$

$$NEL, Mcal / \text{ kg } DM = 1.085 + (0.0124 \times ADL, \text{ as } \% \text{ of } DM) \quad (12)$$

$$ENE, Mcal / \text{ kg } DM = (0.0307 \times TDN) - 0.764 \quad (13)$$

After the NEL and ENE values of the SMS samples were calculated, the values were converted to MJ/kg DM.

***In vitro* true digestibility**

The *in vitro* true digestibility (IVTD) of SMS samples was performed using the Ankom *in vitro* Daisy II (ANKOM Technology, Macedon, NY, USA) fermentation system (Hervás et al., 2004). Rumen fluid (graft) used in the study was collected from a dairy cow freshly slaughtered (Chaudhry & Mohamed, 2012) at a local abattoir (Florya Entegre Et Sanayi, Samsun, Türkiye). According to the statement of the owner farmer, the cow was more than 2.5 years old and fed with a complete feed containing roughage: concentrate ratio of 60:40.

Statistical analysis

The mushroom production panels were used as the experimental unit for statistical analysis in this study. All data were verified for normality and homoscedasticity by the Kolmogorov–Smirnov test and Levene test, respectively. Data expressed as percentages were arcsine transformed before analysis to normalize the distribution. However, we presented the actual values of the data. This study performed statistical analysis in a 2 BS (WS and RS) × 5 FS (WB, BF, RB, WRD, or WRS) factorial design with three repetitions in a completely randomized design. In the general linear model procedure, two-way analysis of variance was performed using the IBM SPSS software package (SPSS v21.0: IBM Corp.). Duncan's multiple comparison test determined the differences between the means, and the differences were considered significant at $P < 0.05$.

Results and Discussion

There was a significant effect of the BS × FS interaction on the DM, OM, ash, EE, and NFE ($P < 0.001$), CP ($P < 0.007$), and CF ($P < 0.005$) contents of SMS (Table 2). The RS+WB- and WS+WB-based SMSs had the highest and lowest DM content among the studied SMSs ($P < 0.05$). Other SMSs were statistically listed as WS+FB > WS+RB > S+WRD > RS+RB > WS+WRD > RS+BF > RS+WRS in terms of DM content ($P < 0.05$). The SMSs comprising WS+WRS, WS+WB or WS+RB had higher OM contents than the other SMSs ($P < 0.05$). In terms of OM content, other SMSs were statistically ranked as WS+WRD > WS+BF > RS+RB > RS+RB = RS-WRD > RS+WDS = RS+BF ($P < 0.05$). The ash content was listed as RS+BF = RS+WRS > RS+WRD > RS+WB > WS+BF > WS+RB = WS+WB = WS+WRS > WS+WRD ($P < 0.05$).

Most studies evaluating agro-industrial residues, including grain byproducts, have focused on changes in the main chemical composition (carbohydrates, protein and fat profiles, and energy values), as in the current study. The novelty of this study is based on the possible co-evaluation of agricultural residues and grain byproducts by applying edible mushroom production technology,

a more sustainable type of solid-state fermentation (Souza et al., 2016). This study revealed that nutrient content, carbohydrate fractions, and forage quality parameters, excluding energy values of SMS, showed significant variations resulting from the interaction between BS and FS. Additionally, our results indicated variation among BSs regarding some parameters depending on BS alone (ADF, DDM, ME, ENE, and TDN) or FS only (ADL and NEL). Indeed, the BF and WRD supplemented-BSs enhanced the feed-nutritional value and *in vitro* digestibility of their SMSs, which can be used as animal feedstock to ensure easy digestibility by ruminants (Hanafi et al., 2018). These results can be explained by the fact that the materials used as BS and FS differ in polysaccharides and nitrogen that king oyster mushrooms need for optimal production and yield (Rinker, 2017). In line with the previous reports (see (Gao et al., 2023) for review), the SMSs evaluated in our study indicated superior nutrient content and digestibility and, as a result, feed value compared to the relative crop straw. Some SMSs (WS+BF, WS+WRD, WS+WRS, WS+RB, and RS+WB) had biological efficiency values of over 50% due to their high productivity (Table 1). These results indicate that SMSs with high biological efficiency might be considered to be sustainable agricultural waste utilization methods (Leong et al., 2022).

The WS+BF-based SMS, which had a similar value to those of the RS+BF, WS+WRS, and RS+WRS in terms of CP content, showed a higher value than those of RS+RB, WS+WRD, RS+WRD, and WS+RB SMSs ($P < 0.05$). The CP content of RS+RB was higher than that of WS+RB ($P < 0.05$). SMSs with BF, which had similar EE contents, had higher EE values than all other SMSs ($P < 0.05$). Similarly, the EE contents of RS+WRD and WS+WB SMSs were higher than those of RS+RB, WS+WRD, RS+WB, WS+WRS and RS+WRS ($P < 0.05$). The WS+RB group also showed a higher EE content than the RS+RB, WS+WRS, and RS+WRS groups ($P < 0.05$). The WS+WB and WS+WRS groups had higher CC values than the other SMS groups, except for the RS+WRS and RS+RB groups ($P < 0.05$). The RS+WRS had a higher CC value than RS+WRD, RS+BF, RS+WB, and WS+WRD ($P < 0.05$). The CC content of RS+RB was higher than those of RS+WB and WS+WRD ($P < 0.05$). The NFE contents of WS+WRD and WS+RB were higher than those of the other SMS groups, except for RS+WB ($P < 0.05$). The NFE content of the RS+WB group was higher ($P < 0.05$) than those of the RS+RB, RS+BF, RS+BU, RS+WRD, WS+BF, and RS+WRS groups, which had similar NFE contents. Regarding NFE, the RS+WRS group had a lower value than the RS+RB and RS+BF groups ($P < 0.05$).

The results regarding the chemical composition of SMSs evaluated in the current study are consistent with literature reports on WS and RS-based SMSs (Khattab et al., 2013; Fazaeli et al., 2014; Sardar et al., 2017; Hanafi et al., 2018). Observed differences in OM or ash contents of SMSs are due to the consumption of OM provided by BSs and FSs by the lignolytic fungus during mushroom production (Khattab et al., 2013; Sardar et al., 2017). Sardar et al. (2017) noted that king oyster mushrooms caused a

significant decrease in cellulose, hemicellulose, and lignin in the compost. Therefore, the reductions in the OM of the WS-BF, WS-BF, and WS-WRD-based SMSs indicate that the polysaccharides and lignin in these SMSs were consumed more by king oyster mushrooms (Arora & Sharma, 2011), a ligninolytic fungi. The differences (Table 1) in the chemical composition (ADF, NDF, and CP contents) of WS and RS may be an explanation that the interaction between BS and FS had a significant effect on most of the examined parameters (Hanafi et al., 2018). Indeed, the milling byproducts used as FS in both composts might have caused differences in the digestibility and the biodegradation of carbohydrates of BSs for fungal mycelia (Arora & Sharma, 2011; Rasib et al., 2015). Thus, there was an increase in nutrients, especially in CP content, but a decrease in cell wall components (Fazaeli et al., 2014; Hanafi et al., 2018).

As seen in Table 3, the effect of the interaction between BS and FS on the NDF ($P<0.001$), CEL ($P<0.002$), HC ($P<0.008$), and NFC ($P<0.001$) contents of SMSs was significant. The WS-based SMS showed a lower ADF content than the RS-based SMS. The ADL content of the SMSs differed only in terms of the FS factor ($P<0.002$). Regardless of BS, the BF-based SMS had a higher ADL content than other FSs, except for RB-based SMS ($P<0.05$). WS+BF had lower values than other SMSs regarding CEL and HC contents ($P<0.05$). The WS-based SMSs showed higher NFC content than the RS-based SMSs. The NFC content of WS+WRD was higher than those of the other SMSs, except for WS+RB ($P<0.05$). The RS+BF, RS+WRD, and RS+WRS groups showed the lowest NFC values, except for WS+RB ($P<0.05$). Among other SMSs, the order was WS+RB = WS+WB > WS+WRS > RS+WB > RS+RB ($P<0.05$).

Table 2: Nutrient content of spent mushroom substrates consisting of wheat or rice straw containing different milling byproducts (% in DM).

BS	FS	DM	OM	Ash	CP	EE	CC	NFE
WS	BF	19.76 ^b	82.41 ^c	17.58 ^d	8.00 ^a	2.53 ^a	35.70 ^{bcd}	36.17 ^{cd}
	WRD	12.91 ⁱ	86.78 ^a	13.21 ^f	7.23 ^{abc}	0.26 ^d	40.82 ^a	38.46 ^b
	WB	11.36 ^j	86.63 ^a	13.36 ^f	6.47 ^{cd}	1.29 ^b	41.09 ^a	37.77 ^b
	RB	19.70 ^c	86.46 ^a	13.53 ^f	6.11 ^d	1.09 ^{bc}	35.91 ^{bcd}	43.34 ^a
	WRS	18.06 ^f	84.32 ^b	15.67 ^e	6.80 ^{cd}	0.64 ^{cd}	32.17 ^c	44.69 ^a
RS	BF	16.01 ^g	77.99 ^f	22.00 ^a	7.67 ^{ab}	2.53 ^a	34.50 ^{cd}	33.29 ^c
	WRD	14.44 ^h	78.29 ^f	21.71 ^a	7.22 ^{abc}	0.17 ^d	39.37 ^{ab}	31.52 ^d
	WB	19.86 ^a	81.47 ^d	18.53 ^c	6.41 ^{cd}	0.26 ^c	34.18 ^d	40.62 ^{ab}
	RB	19.26 ^e	80.01 ^e	19.98 ^b	7.06 ^{bc}	0.66 ^{cd}	38.47 ^{abc}	33.81 ^c
	WRS	19.46 ^d	79.96 ^e	20.03 ^b	6.47 ^{cd}	1.65 ^b	34.91 ^{cd}	36.93 ^{cd}
BS								
	WS	16.36	85.32	14.67	6.92	1.16	37.14	40.08
	RS	17.80	79.54	20.45	6.96	1.05	36.28	35.23
FS								
	BF	17.88	80.20	19.79	7.83	2.53	35.10	34.73
	WRD	13.67	82.53	17.46	7.22	0.21	40.09	34.99
	WB	15.61	84.05	15.94	6.44	0.78	37.63	39.19
	RB	19.48	83.24	16.76	6.58	0.87	37.19	38.57
	WRS	18.76	82.14	17.85	6.64	1.15	33.54	40.81
SEM		0.003	0.043	0.043	0.054	0.057	0.388	0.425
Main effect of								
	BS	<0.001	<0.001	<0.001	0.698	0.341	0.284	<0.001
	FS	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	BS × FS	<0.001	<0.001	<0.001	<0.007	<0.001	0.005	<0.001

WS, wheat straw; RS, rice straw; WB, wheat bran; BF, barley flour; RB, rice bran; WRD, wheat red dog; WRS, wheat reduction shorts; BS, basal substrate; FS, food supplement; BS × FS, the interaction between BS and FS; DM, dry matter, OM, organic matter, CP, crude protein; EE, ether extract; CC: crude cellulose; NFE, nitrogen-free extracts.

Table 3: Cell wall and noncell wall fractions of spent mushroom substrates consisting of wheat or rice straw containing different milling byproducts (% in DM).

BS	FS	NDF	ADF	ADL	CEL	HC	NFC
WS	BF	57.13 ^e	44.93	29.24	15.68 ^b	12.20 ^b	18.75 ^a
	WRD	70.72 ^b	46.80	18.57	28.22 ^a	23.91 ^a	13.51 ^c
	WB	69.98 ^{bc}	45.27	17.58	27.69 ^a	24.71 ^a	13.77 ^b
	RB	66.80 ^c	45.36	20.73	24.63 ^a	21.43 ^a	17.13 ^{ab}
	WRS	63.34 ^d	43.16	14.51	28.65 ^a	20.18 ^a	17.96 ^a
RS	BF	68.95 ^{bc}	48.32	22.14	26.17 ^a	20.63 ^a	3.66 ^e
	WRD	74.23 ^a	49.55	20.69	28.86 ^a	24.68 ^a	1.85 ^e
	WB	71.64 ^{ab}	48.05	20.34	27.71 ^a	23.59 ^a	8.17 ^d
	RB	72.28 ^{ab}	47.72	19.26	28.45 ^a	24.56 ^a	5.07 ^{de}
	WRS	75.00 ^a	49.18	18.67	30.51 ^a	25.82 ^a	2.08 ^e
BS	WS	65.59	45.10 ^b	20.13	24.97	20.48	16.22
	RS	72.24	48.56 ^a	20.22	28.34	23.85	4.16
FS	BF	63.04	46.62	25.69 ^a	20.93	16.41	11.20
	WRD	72.47	48.17	19.63 ^b	28.54	24.29	7.68
	WB	70.81	46.66	18.96 ^b	27.70	24.15	10.97
	RB	69.54	46.54	20.00 ^{ab}	26.54	22.99	11.10
	WRS	69.17	46.17	16.59 ^b	29.58	23.00	10.02
SEM		0.214	0.292	0.610	0.385	0.392	0.228
Main effect of							
	BS	<0.001	<0.001	0.940	<0.001	<0.001	<0.001
	FS	<0.001	0.269	0.002	<0.001	<0.001	<0.001
	BS × FS	<0.001	0.311	0.057	0.002	0.008	<0.001

WS, wheat straw; RS, rice straw; WB, wheat bran; BF, barley flour; RB, rice bran, WRD, wheat red dog; WRS, wheat reduction shorts; BS, basal substrate; FS, food supplement, BS × FS, the interaction between BS and FS; NDF, neutral detergent fiber; ADF: acid detergent fiber, ADL, acid detergent lignin, CEL: cellulose, HC: hemicellulose, NFC: nonfiber carbohydrates.

The polysaccharide and protein contents of SMS are more affected by the level of fungal mycelia (Rasib et al., 2015; He et al., 2016) and moisture (Souza et al., 2016) in an SMS rather than the substrate and supplements used in the mushroom production compost. Unfortunately, we did not determine the mycelium content of SMSs. The moisture content of the fresh SMS is vital in terms of changes in the nutrient concentration and microorganism contamination in the process until presented to animals (Souza et al., 2016). The moisture content of SMSs in the current study was more than 80%, which increases the risk of rapid microbial spoilage (Kwak, Jung, & Kim, 2008). Therefore, preservative quality to enhance feed safety and shelf life must be improved for their effective use as animal feed (Sardar et al., 2017; Hanafi et al., 2018). From this perspective, RS+WB, RS+RB, WS+BF, and WS+RB-based SMSs were more disadvantaged than other SMSs.

The interaction effect of BS by FS on the forage quality indicators (DMI, IVTD, RFV, and RFQ) and the effect of BS on DDM were significant ($P < 0.001$, Table 4). The SMS with WS had a higher DDM than RS ($P < 0.05$). Regarding DMI, WS+BF had the statistically highest value ($P < 0.05$). There was no difference in DMI values between WS+WRD, WS+RB and RS+BF, and their DMI values were higher than those of other SMSs ($P > 0.05$). The RS+WRS and RS+WRD groups, which had similar DMI values, had lower DMI values than the RS+WB, RS+RB, WS+BF, and WS+WB groups ($P < 0.05$). Regarding IVTD, WS+BF showed higher values than other SMSs, except RS+RB ($P < 0.05$). The RS+RB group showed higher IVTD values than the WS+WRS, RS+WRS, RS+BF, and RS+WRD groups ($P < 0.05$). The WS+BF and WS+WRD groups had the highest RFV values ($P < 0.05$). WS+WRS,

which ranked third, had higher RFV and RFQ than the other RS-based SMSs, except for RS+BF ($P<0.05$). The RFV and RFQ of RS+WRS and RS+WRD were lower than those of WS+WB, WS+WB and RS+BF ($P<0.05$).

The ME, ENE, and TDN values of the SMSs evaluated in this study were affected by BS ($P<0.001$), while the NEL value was affected by FS ($P<0.002$, Table 5). Regardless of FS, SMS with WS showed higher ME, ENE, and TDN values than SMS with RS. The SMS with BF had a lower NEL value than other FSs, except for SMS with RB ($P<0.05$), regardless of BS.

Our results confirmed that BS-based SMSs could be suitable for ruminants if supplemented with the right FS (Van Kuijk et al., 2015; Jeznabadi et al., 2017; Sardar et al., 2017). Moreover,

from the perspective of which SMSs are preferred, the quality of the feed, which is closely related to nutrients, especially CP content, digestibility level, and energy values, should be considered (Fazaeli et al., 2014; Uzun, Garipoğlu, & Ocak, 2017; Hanefi et al., 2018). Uzun, Garipoğlu and Ocak (2017) noted that the RFV and ME, as well as CP levels in a feedstuff, are significantly limiting nutritive properties for ruminant animals because forages with high these parameters are more digestible and palatable. The chemical compositions, except for CP content, the IVTD, and energy values of SMSs evaluated in the current study were similar or close to those of some low-quality feedstuffs used as a feed source for farm animals (Jeznabadi et al., 2017; Sardar et al., 2017). However, the CP contents of

Table 4: The forage quality parameters of spent mushroom substrates consisting of wheat or rice straw containing different milling byproducts.

BS	FS	DMI (% of body weight)	DDM (%)	IVTD (%)	RFV	RFQ
	BF	2.10 ^a	53.89	42.99 ^a	87.70 ^a	87.54 ^a
	WRD	1.69 ^c	52.44	34.43 ^d	68.99 ^c	67.87 ^c
WS	WB	1.71 ^c	53.63	37.87 ^{bc}	71.37 ^{cd}	71.09 ^{cd}
	RB	1.79 ^{bc}	53.56	36.20 ^{bcd}	74.59 ^{cd}	74.23 ^{cd}
	WRS	1.89 ^b	55.27	38.36 ^{bc}	81.19 ^b	82.16 ^b
	BF	1.74 ^{bc}	51.25	33.19 ^d	69.15 ^{cd}	67.13 ^{cd}
	WRD	1.61 ^d	50.29	34.78 ^{cd}	63.03 ^e	60.52 ^e
RS	WB	1.67 ^{cd}	51.46	35.41 ^{bcd}	66.83 ^{de}	65.04 ^{de}
	RB	1.66 ^{cd}	51.72	39.82 ^{ab}	66.56 ^{de}	64.97 ^{de}
	WRS	1.60 ^d	50.58	32.31 ^d	62.74 ^e	60.44 ^e
	SEM					
BS	WS	1.84	53.76 ^a	37.97	76.76	76.58
	RS	1.65	51.06 ^b	35.10	65.66	63.62
FS	BF	1.92	52.57	38.09	78.42	77.33
	WRD	1.65	51.37	34.60	66.01	64.19
	WB	1.69	52.54	36.64	69.10	68.07
	RB	1.72	52.64	38.01	70.57	69.60
	WRS	1.74	52.93	35.33	71.96	71.30
SEM		0.005	0.228	0.292	0.357	0.520
Main effect of						
	BS	<0.001	<0.001	<0.001	<0.001	<0.001
	FS	<0.001	0.270	0.003	<0.001	<0.001
	BS × FS	<0.001	0.313	<0.001	<0.001	<0.001

WS, wheat straw; RS, rice straw; WB, wheat bran; BF, barley flour; RB, rice bran; WRD, wheat red dog; WRS, wheat reduction shorts; BS, basal substrate; FS, food supplement, BS × FS, the interaction between BS and FS; DMI, dry matter intake; DDM: digestible dry matter, IVTD: in vitro true digestibility; RFV, relative feed value; RFQ, relative forage quality.

all SMS are lower than the threshold 10% (Hassan & Umar, 2004), which negatively affects microbial protein synthesis in the rumen. Therefore, as previously reported (Hanefi et al., 2018; Madzingira et al., 2021; Leong et al., 2022), the SMSs evaluated in the current study should not be used as suitable as sole feedstuff for ruminants. However, after suitable drying, all SMSs can provide the bulk base required in ruminant mixed rations (Madzingira et al., 2021) or replace a part (up to 15%) of concentrates and forages in diets formulated for sheep (Aldoori et al., 2015) and cattle (Baek et al., 2017) due to improvements in the DMI, the zootechnical performances, and profitability (Leong et al., 2022). Therefore, based on *in vitro* results, the best SMSs (WS-based SMSs or BF- and WRD-supplemented SMSs) need palatability and feeding studies for ruminant nutrition.

The effect of the interaction between BS and FS on CP content depended on the degree to which compost materials with high nitrogen support mycelium growth (Owaid, Abed, & Al-Saeedi, 2017). In terms of the CP content, FSs are more suitable for microorganisms and extracellular enzymes (Rasib et al., 2015) because milling byproducts (i.e., FSs) contain higher levels (more than three times) of CP than BS sources. Indeed, the CP content of the WS and RS were 2.56 and 3.44%, while that of the WB, BF, RB, WRD, and WRS was 12.13, 8.13, 14.50, 14.25, and 12.88%, respectively. In addition, the increase in the CP content of SMS may also be due to the pre-cultivating CP content (Table 1) of mushroom substrates (Rasib et al., 2015), the loss of DM or OM depending on fungal growth (He et al., 2016), and the presence of microorganisms and extracellular enzymes in SMS (Khattab et al., 2013).

Table 5: Energy values of spent mushroom substrates consisting of wheat or rice straw containing different milling byproducts.

BS	FS	ME (MJ/kg DM)	NEL (MJ/kg DM)	ENE (MJ/kg DM)	TDN (%)
	BF	7.16	3.02	3.39	51.31
	WRD	6.91	3.57	3.12	49.18
WS	WB	7.11	3.62	3.34	50.92
	RB	7.10	3.46	3.33	50.82
	WRS	7.39	3.78	3.65	53.33
	BF	6.71	3.93	2.90	47.45
	WRD	6.55	3.46	2.72	46.04
RS	WB	6.75	3.48	2.94	47.76
	RB	6.79	3.54	2.99	48.13
	WRS	6.60	3.57	2.77	46.47
BS	WS	7.13 ^a	3.49	3.36 ^b	51.11 ^a
	RS	6.68 ^b	3.49	2.86 ^a	47.17 ^b
FS	BF	6.93	3.20 ^b	3.14	49.38
	WRD	6.73	3.52 ^a	2.92	47.61
	WB	6.93	3.55 ^a	3.14	49.34
	RB	6.94	3.50 ^{ab}	3.16	49.48
	WRS	6.99	3.67 ^a	3.21	49.90
SEM		0.039	0.032	0.043	0.034
Main effect of					
BS		<0.001	0.967	<0.001	<0.001
FS		0.264	0.002	0.272	0.268
BS × FS		0.308	0.056	0.315	0.313

WS, wheat straw; RS, rice straw; WB, wheat bran; BF, barley flour; RB, rice bran, WRD, wheat red dog; WRS, wheat reduction shorts; BS, basal substrate; FS, food supplement, BS × FS, the interaction between BS and FS; ME, metabolisable energy, NEL, net energy lactation, ENE, estimate net energy, TDN: total digestible nutrients.

The biodegradation rate of lignin in WS (53.76%, Patil et al., 2010) is higher than in RS (46.18%, Jafari et al., 2007). Accordingly, the BSs and FSs in the current study may differ in the ligninolytic properties, as well as the initial lignin content (Šimić et al., 2021). Our ADL results suggested that BF is less conducive to reducing lignin content than other FSs, except for RB. This case may be related to the negligible lignin content of BF (0.21-0.28%, Šimić et al., 2021) compared to other FSs. Because lignin, the main component of ADF, cannot be digested by ruminants, WS-based SMSs, which have lower ADF content, are of relatively high quality, regardless of FS (Patil et al., 2010). This prediction was also supported by the results regarding the CEL and HC contents of the SMSs (Sánchez, 2009; Arora & Sharma, 2011; Šimić et al., 2021). Based on the reduced CEL and HC in WS+BF-based SMS, WS+BF may be more conducive as a feedstuff than other SMSs (Sánchez, 2009; Bento et al., 2014). NFC, found inside the cells of plants, is generally more easily digestible than structural carbohydrates found in plant cell walls (Singh et al., 2012). In our study, the WS as BS and the SMSs with FSs such as BF, WRD and RB had higher IVTD compared to RS and other FSs because these materials had a higher NFC content or lower cell wall components, in agreement with previous studies (Singh et al., 2012).

In the present study, roughage quality parameters such as DMI, IVTD, RFV, and RFQ of the SMS may have been affected by the BS × FS interaction due to different carbohydrate fractions and lignin levels present in these materials (Malinowska & Jankowski, 2021). In the present study, a low CEL, HC, CC, and carbohydrate fraction content, while a high protein and ash content in all SMSs due to the king oyster mushroom growth observed compared to the relative BS and FS, as coincided with earlier reports (Patil et al., 2010; Arora & Sharma 2011; Gao et al., 2023). Moreover, these SMSs had moderate ME, CP, better digestibility, and lower NDF, ADF, CEL, and ADL values than the other SMSs. Based on this information, WS-based SMSs and all SMSs with BF, WRD, and WRS were relatively better forages. This suggestion was similar to that of Kim et al. (2012) and Moradzadeh-Somarin et al. (2021), who reported that WS-based SMS with WB is more common than other lignocellulosic materials. When SMS is used as a feedstuff, this situation may be beneficial regarding feed efficiency because rations prepared for ruminant animals consist of carbohydrates with a chemical and nutritional heterogeneous structure as primary feedstuff (Hanefi et al., 2018). This result supported the idea that using SMS as a forage source could be an inexpensive way to protect the environment and natural resources and benefit animal production (Hanefi et al., 2018; Leong et al., 2022).

Conclusions

Our results indicate that SMS with WS, regardless of FS, and SMS with BF and WRD, regardless of BS, can be a

relatively better forage source. However, according to their quality categories, CP content, and energy values, any of the SMSs examined alone will not be sufficient to meet the needs of ruminant animals. Further research is needed to determine the optimum SMS level that provides the desired animal performance to supplement total mixed ration with dietary SMS.

Author Contribution

Conceptual idea: Ocak, N.; Methodology design: Akdağ A.; Data collection: Bilik B.; Akdağ A.; Data analysis and interpretation: Bilik, B.; Akdağ, A.; and Writing and editing: Ocak, N.; Bilik, B.; Akdağ, A.

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