



## Spent Mushroom Substrate Influences Elk (*Cervus Elaphus Canadensis*) Hematological and Serum Biochemical Parameters

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**ABSTRACT :** The objective of this study was to evaluate the effect of spent mushroom substrate (SMS) derived from *Pleurotus eryngii* on the hematological and biochemical blood properties of elk. A total of 18, two and three-year-old elk were fed three different levels of SMS (0, 15 and 20%) in a corn-wheat bran diet for 80 days. The results indicated significantly high levels of blood monocytes, hemoglobin (Hb), and hematocrit (HCT) in elk fed 15% or 20% SMS ( $p < 0.05$ ) compared to control animals. Serum blood urea nitrogen (BUN) and glucose concentrations were also significantly elevated in elk fed both 15% and 20% SMS. The inclusion of SMS in the elk diet did not affect serum total cholesterol, triglyceride, or low density lipoprotein (LDL)-cholesterol concentrations; however, high density lipoprotein (HDL)-cholesterol concentration was significantly increased in SMS-fed groups. In addition, 20% SMS in the diet increased serum iron and testosterone concentrations in elk. These results indicate that adding SMS to the diet of elk can increase their Hgb, serum BUN, glucose, and HDL-cholesterol concentration; therefore, diets containing SMS may enhance the physiologic condition of elk during growth. (**Key Words :** Spent Mushroom Substrate, Recycling, Blood Components, Elk)

### INTRODUCTION

Interest in the addition of agricultural by-products to animal diets has increased due to increased feed cost and environmental issues. Mushroom substrate, a by-product generated from mushroom production is among the agricultural by-products of interest. In Korea, approximately 300,000 metric tons of spent mushroom substrate (SMS) is produced annually and presents an economic problem due to associated disposal costs (Forestnews, 2011). Therefore, much effort has been spent in an attempt to increase potential applications for SMS in the agriculture industry. To date, SMS has been used mainly for soil amelioration (Brunetti et al., 2009; Marín-Benito et al., 2009; Herrero-Hernández et al., 2011) or as a soil conditioner (Tajbakhsh et al., 2008) and vermiculture bed material (Edwards and Baxter, 1992). Until recently, most studies related to SMS recycling mainly focused on horticulture. Mushroom growth medium is composed of

agricultural materials, including rice straw, saw dust, corn cobs, rice bran, wheat bran, beet pulp, and bean curd, and as such could be a potential feed source for ruminants. Recently, some papers have reported the use of SMS in ruminant diets and have demonstrated the effects of fiber from SMS on ruminants, thereby showing the use of SMS in ruminant diets is possible and should be considered (Lee et al., 2008; Kim et al., 2010; Oh et al., 2010; Xu et al., 2010). Even though some evidence for SMS as a dietary supplement has been confirmed, few data are available, in particular with regard to the biological properties of SMS in ruminant animals. Therefore, the objective of our study was to collect information on SMS as a feed ingredient by measuring hematological and biochemical blood properties in elk that were fed diets which included SMS.

### MATERIALS AND METHODS

#### Animals and sample preparation

A total of 18 two to three-year-old, male elk deer (*Cervus elaphus canadensis*) of similar body weight ( $220 \text{ kg} \pm 8.25$ ) were used as study animals. Six elk each were housed in three wire cages ( $180 \text{ m}^2$ ). An experimental diet, hay (Italian ryegrass), water, and trace mineral salt were

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provided *ad libitum* during the 80-d study period. SMS, from *Pleurotus eryngii* production, was added at levels of 15% and 20% to a basal diet composed mainly of corn, wheat bran, and brewer's grain (Table 1).

At the end of the experiment, blood samples were taken from the jugular vein immediately after anesthesia (1.5 ml Fentazine10; Parnell, New Zealand) to determine hematological and biochemical properties. For hematology, approximately 3 mL of blood in tubes containing K2EDTA (BD Vacutainer®, Plymouth, PL6 7BP, UK) was analyzed immediately after collection. Blood samples for biochemical determinations were placed in serum separator tubes (BD Vacutainer®) and centrifuged at 3,000 rpm for 15 min then stored at -70°C until assayed.

#### Beta-glucan and chemical composition analysis of SMS

Moisture content, crude protein, ether extract, ash content, and crude fiber of SMS were determined according to standard AOAC (2000) methods. Calcium and phosphorus contents were determined by sequential plasma spectrometer (ICP-7510, SHIMADZU, Japan). Gross energy was measured using a bomb calorimeter (PARR 1351, USA).

$\beta$ -glucan levels of lyophilized SMS were determined by

acidic and enzymatic hydrolysis using the mushroom and yeast  $\beta$ -glucan assay according to the manufacturer's protocol (Megazyme, Wicklow, Ireland).

#### Hematological and biochemical analysis

Blood samples for hematology determination were mixed on a roller mixer for 30 min and analyzed with a HemaVet 850 (CDC Technologies, Inc., Oxford, CT). Hematological parameters measured included white blood cells (WBCs), neutrophils, lymphocytes, monocytes, eosinophils, basophils, red blood cells (RBCs), hemoglobin (Hb) hematocrit (HCT), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH).

Serum samples were evaluated using an enzymatic colorimetric assay for total protein, albumin, blood urea nitrogen (BUN), creatinine, glucose, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol, high density lipoprotein (HDL)-cholesterol, low density lipoprotein (LDL)-cholesterol, triglyceride, calcium, phosphorus, sodium, potassium, and iron using a Hitachi 7600 automatic analyzer (Hitachi Co., Japan).

Serum thyroxine and testosterone levels were determined using an electrochemiluminescence immunoassay method (ECLIA) with a Roche Modular Analytics E170 Immunoassay System (Roche Diagnostics GmbH, Mannheim, Germany). Growth hormone and insulin-like growth factor-I (IGF-I) levels were analyzed using radioimmunoassay techniques and a 1470 WIZARD  $\gamma$ -counter (Wallac, Turku, Finland).

#### Statistical analysis

All statistical analyses of the data obtained were performed using the general linear model (GLM) procedure of SAS software (Statistical Analysis System, version 9.2). Statistical differences are expressed at  $p < 0.05$ .

## RESULTS

$\beta$ -glucan is one of the major components extracted from many species of mushroom including *Pleurotus eryngii*. The presence of  $\beta$ -glucan in *Pleurotus eryngii* by-product was identified by an enzyme method as being 199.2 mg/g, and moisture, crude protein, ether extract, crude fiber, ash, and energy of SMS were 42.4%, 8.5%, 2.1%, 5.3%, 4.6% and 2,564 kcal/kg, respectively (Table 2).

No statistically significant differences were observed in WBCs, neutrophils, lymphocytes, eosinophils, basophils, RBC, MCV, or MCH (Table 3). The number of monocytes significantly increased in elk fed a diet containing 15% SMS. The percentage of HCT was higher in 15% and 20% SMS-fed groups as compared to control ( $p < 0.05$ ); however,

**Table 1.** Experimental feed formula and chemical composition

Ingredients	SMS 0%	SMS 15%	SMS 20%
Corn	38.52	30.00	30.00
Spent mushroom substrate	-	15.00	20.00
Wheat bran	19.76	12.00	12.00
Wet brewer's grain	14.00	14.00	11.00
Rice bran	10.00	10.00	8.00
Soybean meal	8.00	9.59	10.03
Perilla meal	5.00	5.00	5.00
Molasses	2.00	2.00	2.00
Calcium phosphate	2.22	1.91	1.47
Vit-min premix <sup>1</sup>	0.50	0.50	0.50
Calculated chemical composition			
Moisture	20.88	29.80	31.04
CP (%)	13.90	13.61	13.48
TDN (%)	63.76	57.00	56.41
Ca (%)	0.80	0.80	0.75
P (%)	0.85	0.79	0.73
C. fat (%)	4.27	3.98	3.92
C. fiber (%)	6.11	7.15	7.45
C. ash (%)	7.15	6.96	6.34
NDF (%)	16.45	16.42	16.76
ADF (%)	6.06	7.44	7.89

<sup>1</sup> Vit. A, 2,500,000 IU; Vit. D<sub>3</sub>, 500,000 IU; Vit. E, 2,000 mg; Mg 3,000 mg; Mn, 4,000 mg; Fe, 5,600 mg; Zn, 1,500 mg; Cu, 375 mg; I, 140 mg; Co, 100 mg; Tu, 350  $\mu$ g.

**Table 2.** Chemical composition of spent mushroom substrate (SMS)

	SMS
Moisture (%)	42.35
Energy (kcal/kg)	2,564
CP (%)	8.46
C. fat (%)	2.09
C. ash (%)	4.58
C. fiber (%)	5.27
Ca (%)	0.69
P (%)	0.76
β-glucan (mg/g)	199.2

no significant differences in the amounts of total protein, albumin, creatinine, bilirubin, AST and ALT were determined. BUN and glucose concentrations were increased in 15% and 20% SMS-fed groups compared to control ( $p < 0.05$ ) (Table 4). The inclusion of 15% or 20% SMS increased HDL-cholesterol by 14% compared to the control diet ( $p < 0.05$ ), but total cholesterol, triglyceride, and LDL-cholesterol were not affected (Table 5). Serum iron concentration was found to be significantly elevated in the 20% SMS group compared to those groups fed 15% SMS or no SMS ( $p < 0.05$ ) (Table 6). Inclusion of SMS did not affect calcium, phosphorus, sodium, or potassium concentrations. Additionally, the SMS feeding regime did not affect serum growth hormone, IGF-I, and thyroxine concentrations in the animals. A significant increase in testosterone level was found in those fed 20% SMS

**Table 3.** Effect of spent mushroom substrate (SMS) on hematological properties of elk

	Control	SMS 15%	SMS 20%	SEM <sup>2</sup>
WBCs <sup>1</sup> (K/μl)	4.19	4.24	4.04	0.12
NE (K/μl)	2.45	2.29	2.02	0.14
LY (K/μl)	1.33	1.02	1.34	0.06
MO (K/μl)	0.22 <sup>b</sup>	0.35 <sup>a</sup>	0.26 <sup>ab</sup>	0.02
EO (K/μl)	0.18	0.54	0.39	0.07
BA (K/μl)	0.02	0.04	0.02	0.01
RBCs (M/μl)	6.88	7.04	7.28	0.13
Hb (g/dl)	8.38 <sup>b</sup>	9.48 <sup>a</sup>	9.56 <sup>a</sup>	0.22
HCT (%)	27.08 <sup>b</sup>	29.5 <sup>ab</sup>	30.98 <sup>a</sup>	0.61
MCV (fL)	39.68	41.86	42.60	0.87
MCH (pg)	12.25	13.48	13.14	0.29

<sup>1</sup> WBCs = White blood cells, NE = Neutrophils, LY = Lymphocytes, MO = Monocytes, EO = Eosinophils, BA = Basophils, RBCs = Red blood cells, Hb = Hemoglobin, HCT = Hematocrit, MCV = Mean corpuscular volume, MCH = Mean corpuscular hemoglobin.

<sup>2</sup> Standard error of the mean.

<sup>ab</sup> Means in the same row with different superscripts differ significantly ( $p < 0.05$ ).

**Table 4.** Effect of spent mushroom substrate (SMS) on the biochemical properties of elk

	Control	SMS 15%	SMS 20%	SEM <sup>2</sup>
Total protein (g/dl)	5.9	5.9	5.8	0.05
Albumin (g/dl)	3.3	3.2	3.4	0.03
BUN <sup>1</sup> (mg/dl)	25.4 <sup>b</sup>	30.4 <sup>a</sup>	32.2 <sup>a</sup>	0.91
Creatinine (mg/dl)	1.2	1.3	1.4	0.03
Glucose (mg/dl)	108.4 <sup>b</sup>	143.6 <sup>a</sup>	158.0 <sup>a</sup>	7.43
Bilirubin (mg/dl)	0.1	0.1	0.1	0.00
AST (IU/L)	64.6	69.4	65.0	2.54
ALT (IU/L)	34.8	33.6	36.2	2.79

<sup>1</sup> BUN = Blood urea nitrogen, AST = Aspartate aminotransferase, ALT = Alanine aminotransferase.

<sup>2</sup> Standard error of the mean.

<sup>ab</sup> Means in the same row with different superscripts differ significantly ( $p < 0.05$ ).

compared to the control and 15% SMS groups ( $p < 0.05$ ) (Table 7).

## DISCUSSION

Numerous studies have shown that mushroom consumption has various beneficial effects including immunomodulatory, hypocholesterolemic, and anti-tumor influences on animals and humans (Yoshioka et al., 1985; Fukushima et al., 2000; Lull et al., 2005). The biologically

**Table 5.** Effect of spent mushroom substrate (SMS) on serum lipid properties of elk

	Control	SMS 15%	SMS 20%	SEM <sup>1</sup>
Total cholesterol (mg/dl)	74.2	84.8	90.8	3.28
Triglyceride (mg/dl)	7.4	6.2	10.0	1.15
HDL-cholesterol (mg/dl)	70.2 <sup>b</sup>	80.0 <sup>a</sup>	80.8 <sup>a</sup>	2.12
LDL-cholesterol (mg/dl)	10.8	11.4	15.0	1.19

<sup>1</sup> Standard error of the mean.

<sup>ab</sup> Means in the same row with different superscripts differ significantly ( $p < 0.05$ ).

HDL = High density lipoprotein, LDL = Low density lipoprotein.

**Table 6.** Effect of spent mushroom substrate (SMS) on serum mineral content of elk

	Control	SMS 15%	SMS 20%	SEM <sup>1</sup>
Calcium (mg/dl)	8.7	8.8	8.7	0.05
Phosphorus (mg/dl)	7.0	7.3	7.3	0.13
Sodium (mmol/L)	139.6	139.8	141.2	0.59
Potassium (mmol/L)	4.3	4.4	4.3	0.04
Iron (μg/dl)	187.8 <sup>b</sup>	180.0 <sup>b</sup>	197.0 <sup>a</sup>	0.94

<sup>1</sup> Standard error of the mean.

<sup>ab</sup> Means in the same row with different superscripts differ significantly ( $p < 0.05$ ).

**Table 7.** Effect of spent mushroom substrate (SMS) on elk serum hormone levels

	Control	SMS 15%	SMS 20%	SEM <sup>1</sup>
Growth hormone (ng/ml)	0.025	0.032	0.029	0.005
Insulin-like growth factor-I (ng/ml)	633.7	825.3	682.0	40.92
Thyroxine (µg/dl)	4.3	4.3	4.7	0.16
Testosterone (ng/ml)	0.2 <sup>b</sup>	0.8 <sup>ab</sup>	1.0 <sup>a</sup>	0.15

<sup>1</sup> Standard error of the mean. <sup>a,b</sup> Means in the same row with different superscripts differ significantly ( $p < 0.05$ ).

active compounds responsible for these actions are  $\beta$ -glucans, protein-bound polysaccharides found in the cells of mushrooms. The primary objective of the current study was to confirm the presence of  $\beta$ -glucan in *Pleurotus eryngii* by-products since no studies measuring the amount of  $\beta$ -glucan in SMS have yet been reported. In the current study, the level of  $\beta$ -glucan of *Pleurotus eryngii* by-product was 19.9%, which is relatively 80% of that measured by Choi et al. (2010) in edible *Pleurotus eryngii* mushrooms at 24.2%. Thus, various positive effects from a nutritional-physiological aspect could be expected from including SMS in animal diets.

In this study, elk fed diets containing SMS revealed higher levels of monocytes compared to elk fed control diet. Monocytes are generally known as tissue macrophages, which comprise 5-10% of WBCs. Many studies have investigated the immune-enhancing effects of mushrooms. In particular, mushroom  $\beta$ -glucans enhance the function of macrophages, the immune system, and resistance to microbial infections (Volman et al., 2008).  $\beta$ -glucans are known as the primary components responsible for the physiological effects of mushrooms (Mizuno et al., 1998; Nakajima et al., 2002; Diyabalanage et al., 2008). The findings of our current study imply that SMS inclusion in the diet may have modulating effects on the immune system by activating monocytes, although no significant differences were seen in WBC counts between the SMS groups and control.

Hb, HCT, and iron levels in elk fed diets with SMS were significantly increased. Mushrooms have been recognized as a source of iron which is essential for the synthesis of Hb and oxygenation of RBCs. These results appear to be consistent with another recent study (Regula et al., 2010), which found that feeding products with 10% and 20% dried shiitake (*Lentinula edodes*) to rats with iron deficiency resulted in an increase in blood Hb concentration and serum and liver iron levels. Regula et al. (2010) also found that the iron in dried shiitake mushrooms had equivalent bioavailability as the iron form ferrous gluconate (iron supplement). Increased Hb, HCT, and iron levels in this study due to the inclusion of dietary SMS may be related to the high iron bioavailability of SMS. Thus, SMS inclusion in elk diet may result in elevated Hb of RBCs and serum iron levels.

The differences in BUN and glucose levels found with the addition of SMS to the diet may be considered an indicator of nutrient status. In ruminants, BUN can be influenced by quantity of dietary protein, level of feed intake, and protein degradability in the rumen (Karnezos et al., 1994). Blood glucose level is used as an indicator of ruminant energy status. In the current study, elk offered SMS had higher BUN and glucose concentrations, and these results may be attributed to higher nitrogen (N) and energy consumption due to increased feed intake of 10% (15% SMS group) and 17% (20% SMS group), as compared to the control group (data not shown).

To evaluate the functional ability of the liver, concentrations of bilirubin, ALT, and AST were measured in the blood and were found to be similar in all groups. Generally, increased activity of bilirubin, ALT, and AST are evident in liver injury. Our results indicate that SMS can be added up to 20% in elk diet without any significant toxicity.

Serum lipid metabolites in relation to mushrooms and their extracts have been extensively studied. The addition of mushrooms affected serum triglyceride and cholesterol concentrations and effectively prevented the progress of hypercholesterolemia and cholesterol accumulation in liver induced by a high cholesterol diet in rats (Bobek et al., 1995; Fukushima et al., 2001). Results obtained in the present study show that SMS increases serum HDL-cholesterol, regardless of total cholesterol, LDL-cholesterol, and triglyceride.

The present study also indicates that SMS increases serum testosterone levels of elk. We found no studies regarding the influence of SMS, or mushrooms, or their extracts, on testosterone levels in elk or ruminants. However, Grube et al. (2001) identified that shiitake, white button mushroom, portobello, crimini, and baby button mushrooms had the most potent inhibitory effects against aromatase activity which converts testosterone into estrogen in humans.

There is currently a lack of useful information regarding SMS in elk, and previously reported functions of SMS in other species may not be directly compared. However, the findings of the current study support that of previous research (Lee et al., 2008; Oh et al., 2010) which determined that by-products of *Pleurotus eryngii* or *Pleurotus ostreatus* could be used as ruminant feed without

any harmful effects on eating behavior and blood metabolites in cattle. Silvana et al. (2006) and Adamovic et al. (1998) reported that biodegradation of straw with *Pleurotus ostreatus* increased its nutritional value and digestibility in ruminant diets.

The results of the current study confirmed the applicability of SMS as a feed ingredient in elk diets. Further study with more focus on growth performance is therefore suggested.

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