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Hematological and Serum Biochemical Parameters of Korean Native Goats Fed with Spent Mushroom Substrate

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ABSTRACT

Korean native black goats were used to investigate the additive effect of spent mushroom substrate (SMS) derived from *Pleurotus eryngii* on the hematological and biochemical properties of blood during growth. Forty five goats (five and six months old, 16.2±1.39 kg) were fed three different levels of SMS (0, 15 and 20%) in a diet based on corn-brewer's grain for 6 weeks. Goats fed a 15 % SMS diet for 6 weeks had significantly higher blood white cell (WBCs) and lymphocyte (LY) counts than goats fed a 20% SMS diet or controls. Most serum biochemical parameters including the total protein, albumin, Blood Urea Nitrogen (BUN), creatinine, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum total cholesterol, triglyceride and High Density Lipoprotein (HDL)-cholesterol levels were not influenced by SMS- based diet. However, Low Density Lipoprotein (LDL)-cholesterol levels were significantly lower in the 15 and 20% groups than in controls. SMS in diet did not affect serum concentrations of calcium, phosphorous, sodium, potassium, or iron. On the other hand, 15% SMS increased serum IGF-I concentrations. These results show that diets containing SMS can increase blood WBC, LY counts, IGF-I concentrations and decrease serum LDL-cholesterol concentrations which SMS suggests that SMS in diet can affect the physiologic conditions of growing goats positively.

Key words: Spent mushroom substrate, *Pleurotus eryngii*, recycling, blood components, goats

INTRODUCTION

Pleurotus eryngii (king oyster mushroom) is one of the most popular types of mushroom produced and eaten in Korea. In the past, mushrooms were viewed as medicines rather than foods and in traditional Chinese medicine, mushrooms are considered to have immune, antibacterial, detoxifying and diuretic effects (Moradali *et al*., 2007; Lee *et al*., 2009). Furthermore, recent studies indicated that mushrooms have anticancer and immune system revitalizing effects and that they lower of blood cholesterol levels (Wasser, 2002; Enman *et al*., 2007; Chung *et al*., 2010). The contents of mushroom differ but almost all species contain β-glucan as a main ingredient. β-Glucan

is a polysaccharides, fibrous material that is difficult to digest and is known to prevent arteriosclerosis, cardiac disorders and diseases and reduce high blood pressure by lowering cholesterol levels (Tam *et al*., 1986; Strong *et al*., 2005; Vetvicka and Yvin, 2004). β-Glucan is also known to prevent the proliferation and recurrence of cancer because it speeds up the functions of immunocytes (Takaku *et al*., 2001; Nilsson *et al*., 2004).

Spent Mushroom Substrate (SMS) is regarded as an agricultural waste product in the mushroom industry. The characteristic of media used for cultivation depend on mushroom type and cultivation method. However, Park *et al*. (2012) suggested that the SMS media can be used as a feed ingredient in ruminants and they are composed of rice bran, wheat bran, beet pulp, bean curd, corn cob, rice straw and saw dust. Furthermore, only 20% of media are utilized by the cultivation process (Williams *et al*., 2001). SMS is considered no more than waste by mushroom farmers but as an excellent feed source by stock farmers. Furthermore, because SMS production has been on the increase due to the mechanization of the growing process and development of automation technology, it has been considered as an appropriate feed material. The total production of SMS in Korea is estimated at 300,000 metric tons annually (Park *et al*., 2012) and this is expected to increase continuously in line with mushroom consumption.

SMS is used as gardening compost or soil conditioner in the West (Medina *et al*., 2009), but little information is available on its used as a feed material for goats. Therefore, this study was undertaken to determine the hematological and serum biochemical parameters after feeding SMS to goats in feed to investigate its potential use as feed material.

MATERIALS AND METHODS

Experimental design and blood sampling: Animal management and the experimental procedures were performed in accordance with the guidelines for the care and use of experimental animals issued by the Korean National Institute of Animal Science. Forty five seven-month-old, male Korean native black goats (*Capra aegagrus hircus*) with an average body weight of 16.2±0.35 kg were allotted to three groups with three replications of five goats for six weeks. Goats divided into wire pens (45 m^2) were fed an experimental diet in addition to hay (rice straw) and water *ad libitum* for 6 weeks. *Pleurotus eryngii* by-products (SMS) were added at levels of 0, 15 or 20% to a basal diet composed mainly of corn, brewer's grain and wheat bran (Table 1). At the end of the experiment, blood samples were taken from eight goats per group after a 12 h fasting period to measure hematological and biochemical properties. Approximately 3 mL of blood in tubes containing K2EDTA (BD Vacutainer®, Plymouth, PL6 7BP, UK) was collected and analyzed immediately for hematology. Blood samples for biochemical assessments were placed into vacuum tubes (BD Vacutainer®) and centrifuged at 3,000 rpm for 15 min. The separated sera were then stored at-70°C until the required assay was performed.

Beta-glucan and chemical composition analysis of SMS: Moisture content, crude protein, ether extract, ash content and crude fiber in SMS were determined according to the methods described by AOAC (2000) and a sequential plasma spectrometer was used to determine calcium and phosphorus contents (ICP-7510, SHIMADZU, Japan). Gross energy was measured using a bomb calorimeter (PARR 1351, USA) and β-glucan levels in SMS were determined by acidic and enzymatic hydrolysis, according to the manufacturer's instructions (Megazyme, Wicklow, Ireland).

Hematological and biochemical analysis: For hematology assessments, blood samples placed on a roller mixer for 30 min and then analyzed using an automated hematology analyzer (Forcyte

Table 1: Formulation and calculated chemical composition of experimental diet (as-fed basis)

	SMS		
Ingredients	0%	15%	20%
Corn	39.60	35.25	31.12
Spent mushroom substrate	-	15.00	20.00
Wet brewer's grain	20.75	16.25	15.91
Wheat bran	15.00	15.00	15.00
Soybean meal	10.16	9.33	12.04
Rice bran	7.39	-	-
Perilla meal	3.25	5.62	2.45
Limestone	1.35	1.05	0.98
Molasses	2.00	2.00	2.00
Vit-min premix[1]	0.50	0.50	0.50
Nutrieut compositiou			
Moisture	25.06	26.86	28.04
CP	13.80	13.80	13.80
TDN	61.70	61.70	61.70
Ca	0.60	0.60	0.60
P	0.40	0.40	0.40
C. Fat	3.78	2.63	2.57
C. Fiber	5.57	5.68	5.33
C. Ash	5.55	4.55	4.53
NDF	15.02	15.40	16.32
ADF	5.85	6.93	7.79

[1]Vit. A: 2,500,000 IU, Vit. D_3: 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 μg

Hematology Analyzer, Oxford Science, Oxford, CT). The measured hematological parameters were; total white blood cells (WBCs), neutrophils (NE), lymphocytes (LY), monocytes (MO), eosinophils (EO), basophils (BA), red blood cells (RBCs), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH).

Total protein, albumin, Blood Urea Nitrogen (BUN), creatinine, glucose, 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 were determined using serum samples and a Hitachi 7600 automatic analyzer (Hitachi Co., Japan).

In addition, radioimmunoassay techniques and a 1470 WIZARD α-counter were used to determine insulin-like growth factor-I (IGF-I) levels in serum (Wallac, Turku, Finland).

Statistical analysis: All data collected were analyzed using the general linear model procedure in SAS version 9.2 (Statistical Analysis System). Differences between treatments were determined using Duncan's multiple range test. Statistical significance was accepted for p values of $p<0.05$.

RESULTS

Chemical composition of SMS: The mean β-glucan levels in the *Pleurotus eryngii* SMS was found to be 199.2 mg g^{-1} and levels of moisture, crude protein, crude fat, crude fiber, crude ash and energy were 42.35, 8.46, 2.09, 5.27, 4.58% and 2,564 kcal kg^{-1}, respectively (Table 2).

Table 2: Chemical composition of spent mushroom substrate (SMS)

Parameter (%)	SMS
Moisture	42.35
Energy (kcal kg^{-1})	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^{-1})	199.2

Table 3: Effects of spent mushroom substrate (SMS) on hematological properties of goats

		SMS		
Parameter	Control	15%	20%	SEM
WBCs (K μL^{-1})	11.08^{b}	15.03^{a}	11.27^{b}	0.70
NE (K μL^{-1})	3.07	4.84	3.75	0.39
LY (K μL^{-1})	6.79^{b}	8.55^{a}	6.38^{b}	0.37
MO (K μL^{-1})	1.03	1.41	0.98	0.08
EO (K μL^{-1})	0.13	0.16	0.12	0.01
BA (K μL^{-1})	0.05	0.08	0.04	0.01
RBCs (K μL^{-1})	18.36	17.98	17.78	0.28
Hb (g dL^{-1})	14.23	14.11	13.65	0.16
HCT (%)	31.29	30.46	30.37	0.38
MCV (fL)	17.08	17.05	17.12	0.22
MCH (pg)	7.77	7.87	7.69	0.08

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, Means in the same row with different superscripts are significantly different at $p<0.05$

Hematological parameters of SMS: WBC and LY counts were significantly higher (15.03 and 8.55 K μL^{-1}, respectively) in goats fed 15% SMS for 6 weeks than in goats fed 20% and controls. However, goats fed SMS supplemented diet showed no significant differences in blood NE, MO, EO and BA. In addition, no statistically significant differences were observed between goats fed SMS and goats not fed SMS in terms of RBC, Hb, HCT, MCV and MCH levels (Table 3).

Serum biochemical parameters of SMS: No significant differences in serum total protein, albumin, BUN, creatinine, glucose, AST and ALT levels were found between SMS groups and control group (Table 4).

The concentration of LDL-cholesterol was lower in the 15 and 20% SMS groups (26.60 and 27.90 mg dL^{-1}, respectively) than in the control group (39.11 mg dL^{-1}) ($p<0.05$) but no statistically significant differences in the levels of triglyceride, total cholesterol and HDL-cholesterol were detected (Table 5).

Serum mineral and IGF-I concentrations are presented in Table 6. The addition of 15% SMS was found to increase mean IGF-I concentration by 31.6 % versus the control diet ($p<0.05$) but serum calcium, phosphorus, sodium and potassium concentrations were not affected by feeding SMS.

Table 4: Effect of spent mushroom substrate (SMS) on the serum biochemical properties of goats

		SMS		
Parameter	Control	15%	20%	SEM
Total protein (g dL^{-1})	6.83	6.58	6.61	0.06
Albumin (g dL^{-1})	3.41	3.30	3.29	0.03
BUN (mg dL^{-1})	22.78	23.00	23.30	0.59
Creatinine (mg dL^{-1})	0.50	0.52	0.48	0.02
Glucose (mg dL^{-1})	81.00	81.90	79.00	2.46
AST (IU L^{-1})	98.44	104.40	93.10	6.11
ALT (IU L^{-1})	19.33	19.70	20.30	1.00

BUN: Blood urea nitrogen, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, Means in the same row with different superscripts are significantly different at $p<0.05$

Table 5: Effects of spent mushroom substrate (SMS) on serum lipid properties of goats

		SMS		
Parameter	Control	15%	20%	SEM
Triglyceride (mg dL^{-1})	14.78	9.80	10.80	1.13
Total cholesterol (mg dL^{-1})	119.33	100.50	99.50	4.47
HDL-cholesterol (mg dL^{-1})	71.56	67.90	66.10	1.94
LDL-cholesterol (mg dL^{-1})	39.11^{a}	26.60^{b}	27.90^{b}	2.30

[1]HDL: High density lipoprotein, LDL: Low density lipoprotein, Means in the same row with different superscripts differ significantly at $p<0.05$

Table 6: Effects of spent mushroom substrate (SMS) on serum mineral content and IGF-I levels in goats

		SMS		
Parameter	Control	15%	20%	SEM
Calcium (mg dL^{-1})	9.29	9.00	9.27	0.05
Phosphorus (mg dL^{-1})	8.68	8.12	7.78	0.19
Sodium (mM L^{-1})	145.44	145.30	146.70	0.32
Potassium (mM L^{-1})	5.62	5.29	5.40	0.10
Iron (μg dL^{-1})	195.89	187.10	173.30	7.61
IGF-I (ng mL^{-1})	312.79^{a}	411.56^{a}	360.34^{ab}	15.73

IGF-I: Insulin-like growth factor-I, Means in the same row with different superscripts are significantly different at $p<0.05$

DISCUSSION

Mushrooms were mainly consumed for health purposes for centuries and the biologically active components responsible are called β-glucans (Mizuno *et al*., 1998; Nakajima *et al*., 2002; Diyabalanage *et al*., 2008). Health benefits in relation to mushrooms or their extracts have been extensively reported, but the physiological function of SMS in goats has hardly been reported. The first objective of the present study was to determine the amount of β-glucan in *Pleurotus eryngii* SMS. In the current study, *Pleurotus eryngii* SMS contained 19.9% β-glucan, which reaches relatively 80% of that of edible *Pleurotus eryngii* mushrooms measured by Choi *et al*. (2010). The hypothesis of this study was that the addition of SMS to the goat diet might have positive effects on hematological and serum biochemical parameters. In this study, goat fed a diet containing SMS showed higher WBC and lymphocyte counts than goats fed a control diet. WBC are a major part of the body's immune system and lymphocytes are small white blood cells that play a major role in

defending the body against infectious diseases. Research has shown that β-glucan effectively enhances the immune system by activating White Blood Cells (WBC) including the macrophages, T cells and natural killer (NK) cells (Lindequist *et al*., 2005). A research carried out using β-glucans extracted from fungi showed that these substances act by stimulating neutrophils, eosinophils, monocytes, macrophages and NK cells via specific receptors (Fortes *et al*., 2006). Nevertheless, the detailed modes of action of these polysaccharides have not been determined, although they may regulate some aspects of the humoral and/or cellular components of the immune system (Sullivan *et al*., 2006). Present findings imply that the addition of SMS to diet has a modulating effect on the immune system associated with the activation WBCs and lymphocytes, although no significant differences were found between the 15 and 20% SMS groups and the control group in terms of NE, MO, EO and BA numbers.

To assess the functional abilities of livers and overall health status, concentrations of total protein, albumin, BUN, creatinine, ALT and AST in serum were measured, but they were found to be similar in the study groups. Generally, elevated ALT and AST activities indicate abnormalities in liver cells (Mera *et al*., 2008). However, our toxicological analysis, performed using serum biochemical assays, revealed no suggestion of liver injury in goats fed SMS. Accordingly, our results indicate that SMS can be added to diet at up to 20% without inducing significant toxicity.

In the present study, a significant decrease in serum LDL-cholesterol levels but no significant changes in triglyceride, total cholesterol and HDL-cholesterol concentrations were observed in the SMS groups after 6 weeks of supplementation. Mushrooms have been extensively studied in the context of lipid metabolism and it has been reported that the β-glucans found in grains, such as, oats and barley, are effective at lowering elevated total and LDL-cholesterol levels. Furthermore, the addition of mushrooms to diet has been reported to affect serum triglyceride and cholesterol levels and to prevent the progress of hypercholesterolemia and cholesterol accumulation in liver in rats and hamsters fed induced by a high cholesterol diets (Bobek *et al*., 1995; Cheung, 1998; Fukushima *et al*., 2001; Xu *et al*., 2008). When used for treating high cholesterol levels, it is believed that β-glucans decreases the gastrointestinal absorption of cholesterol and lipids from food (Rahar *et al*., 2011). The results obtained from goats in the present study show that SMS reduces serum LDL-cholesterol levels, but not total cholesterol, HDL-cholesterol, or triglyceride levels.

The present study also indicates that SMS increased serum IGF-I hormone levels in goats. IGF-I is a polypeptide consisting of 70 amino acids with a structure similar to proinsulin. Furthermore, IGF-I performs a crucial function during vertebrate growth and affect several important metabolic processes associated with the growth and differentiation of cells (Froesch *et al*., 1985; Olivecrona *et al*., 1999). We could not find any information on the influence of SMS, or mushrooms, or their extracts, on IGF-I levels in ruminants. However, non-starch polysaccharides, such as, β-glucans may act via a route involving insulin and IGFs, because higher serum levels of IGF-1 are associated with diet (Heald *et al*., 2003). Although these studies were conducted in humans, our results suggest that SMS increases IGF-I hormone levels in goat serum.

There is currently no information to suggest that SMS in diet is associated with physiologic effects in goats and previously reported effects of mushrooms and β-glucans in other experimental animal may not be directly comparable. However, the findings of the present study are supported by previous studies (Lee *et al*., 2008; Oh *et al*., 2010), in which *Pleurotus eryngii* or *Pleurotus ostreatus* by-products were found to be suitable as ruminant feed and that they had no any negative effects on feeding behavior or blood components. Croan (2004) reported that *Pleurotus* mushrooms have excellent nutritional value and digestibility and that they contain most essential amino acids and have high dietary fiber, mineral and vitamin contents.

CONCLUSIONS

This study has identified for the first time that the mean β-glucan amount in the *Pleurotus eryngii* SMS was 199.2 mg g^{-1}. Goats fed a 15 % SMS diet for 6 weeks had significantly higher Blood White Cell (WBCs) and lymphocyte (LY) counts than goats fed a 20% SMS diet or controls. In addition, 15% SMS increased serum IGF-I concentrations. The results of the present study indicate that SMS is a suitable feed ingredient for goats. In field studies on the growth performance of SMS in goats are suggested.

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