Feed Hygiene and Meat Safety of Cattle Fed Processed Rice Hulls-bedded Broiler Litter*

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ABSTRACT : A study was conducted to determine the safety of feeding processed broiler litter (BL) to beef cattle. The litter was processed by deepstacking, ensiling and composting. The health issues addressed relevant to the safety of feeding litter included pathogenic bacteria, mycotoxins, heavy metals, medicinal drugs and pesticide residues. Exp. 1 evaluated the feed hygiene of processed rice hulls-bedded BL. The presence of pathogenic bacteria in BL was determined before and after deepstacking. A total of 21 BL samples were collected over a 3-year period of commercial and experimental production of BL for beef cattle. Exp. 2 evaluated the safety of meat of cattle fed deepstacked BL. In Exp. 1, there were no pathogenic bacteria, such as coliform, *E. coli*, *E. coli O157:H7*, *Salmonella*, *Listeria* and *Proteus*, in deepstacked BL. Levels of heavy metals (Cu, Fe, Mn and Zn) and toxic heavy metals (As, Pb, Cd and Hg) were lower than the commercial feed tolerances. Aflatoxin, medicinal drug and pesticide residues were detected at extremely low levels. In Exp. 2, the meat of the BL-fed animals exhibited few differences in all analyzed items from that of the control group, showing safety from pathogenic microorganisms and heavy metals. When BL was withdrawn for 14 days prior to slaughtering the BL-fed cattle, no medicinal drug residues were detected in the meat. Pesticides in the tissues of either group of animals were much lower than the tolerances. In conclusion, processed rice hulls-bedded BL and the meat of cattle fed BL were safe from the potential hazards of pathogenic bacteria, heavy metals, aflatoxin, medicinal drugs and pesticide residues. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 11 : 1509-1517)***

Key Words : Broiler Litter, Rice Hulls, Feed, Meat, Hygiene, Safety

INTRODUCTION

Since the importation of beef and live cattle was permitted in Korea in 2001, cattle farmers have tried to strengthen their competitiveness to lower cost of imported beef by improving the quality of their beef and by lowering feed costs.

In Korea, about 0.5 million tons of BL are produced annually (Park et al., 2000). More than 80% of the BL is assumed collectable and usable as feed. For the last several decades, BL has been successfully used as feed for ruminant animals worldwide. In Korea, BL has effectively substituted for cereal by-products and rice straw in diets for growing beef cattle and dairy replacement heifers (Kwak et al., 2000).

Before feeding, BL should be processed in order to destroy pathogenic microorganisms. In US where BL has been widely used as feed, deepstacking, ensiling, artificial dehydration, and extrusion-pelletizing have been used to process BL (Carter, 2002). Potential hazards to the animals fed BL might include pathogenic microorganisms, toxic fungi, residues of pesticides and medicinal drugs, and heavy metals (Fontenot, 2001).

Ruffin and McCaskey (1990) reported that pathogenic microorganisms inoculated into BL were killed when the litter was deepstacked for 5 days, and the risk of pathogens was generally eliminated after 20 days of deepstacking. The total aerobic bacterial count of unprocessed BL is reported to be about 1 billion bacteria per gram (Lu et al., 2003). Bacteria of the genus *Enterococcus* and the coliform group of bacteria account for 0.1 and 0.01%, respectively, of the total aerobic bacterial count. Lactobacilli and *Salinococcus* spp. were reported to be the dominant bacteria in BL (Lu et al., 2003).

Antibiotics are incorporated into broiler poultry diets to improve broiler performance. Some of the antibiotics in their active forms are excreted by the broiler and appears in the BL. The antibiotics remaining in the excreta may be decomposed by fermentative microorganisms (Ruffin and McCaskey, 1990). However, any systematic research on the exact degree of decomposition for each antibiotic seems to be absent. With regard to fungi, Crickenberger and Goode (1996) reported that during the ensiling or deepstacking of BL, inappropriate moisture content encouraged the generation of toxic fungi. In the US, the health of cattle fed BL, and the safety of the meat derived from these animals were steadily reviewed in relation to potential chemical residue problems that might be associated with the feeding of BL (McCaskey and Anthony, 1979; Doctorian and Evers, 1997; Davis, 2002).

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Bedding materials used in broiler houses are mainly sawdust, wood shavings, wood chip, straw, peanut hulls, corn cobs and processed paper (Fontenot, 2001; Lacy, 2002). Unlike the US, rice hulls are used as a bedding material in broiler houses in most of the Asia's rice farming regions including Korea. Especially in Korea, a majority of BL is removed from broiler poultry houses more than 3 times a year, while that in the US BL is removed only once or twice a year. Thus, the bedding component in BL in the Asian region may be higher than that in the US. Particularly, its physical and chemical nature is markedly different from that of the US (Kwak et al., 2004). Such differences in physical and chemical properties may appreciably affect the feed value of the processed products. Although BL has been used as ruminant feed in many Asian countries, there is virtually no extensive research on hygienic safety of processed rice hulls-bedded BL and the safety of food products derived from animals fed BL. It has been known that BL can be stored without conspicuous change in its composition for up to 5 years after fermentation (Ruffin and McCaskey, 1990). Litter can be pelletized to improve its handling, transportation and storage, but there is no available knowledge on the effect of storage time on BL quality.

In recent years there has been a growing awareness about beef safety which has led to the development of the 7-step Hazard Analysis Critical Control Point (HACCP) plan. Hygienic certification based on the HACCP plan is required from food production to process. Thus, it seems that more comprehensive clarification on the aspects of possible residues of pesticides and medicinal drugs in the beef is lacking worldwide. Actually, evaluation of hygiene is the prerequisite to ensure the successful use as feed of rice hulls-bedded BL produced in northeast Asian regions.

Accordingly, this study was conducted to verify the hygienic safety of processed rice hulls-bedded BL from pathogenic microorganisms, heavy metals, mycotoxins, medicinal drugs, and pesticides, to evaluate the hygiene of BL pellets stored for a long period and to ascertain the hygienic safety of beef from cattle fed BL.

MATERIALS AND METHODS

Exp. 1: Hygienic safety of processed BL

For the microbial assay, BL was processed by deepstacking approximately 250 tons of litter inside a sheltered trench silo (30 m length×5.7 m width×2.5 m depth) for approximately one month. The method of deepstacking was described in detail in a previous study (Kwak et al., 2001). Totally 3 trials were made in sequence. For each trial, the BL stack temperature was measured using thermocouples inserted at the center (1 m deep from the upper surface) of the stack. Samples were taken in triplicate before and after deepstacking. The maximum BL stack temperature was determined to be over 50°C.

A total of 21 BL samples were collected over a three-year period for analysis of heavy metals, mycotoxins, and residues of medicinal drugs and pesticides. Ten of these samples were collected from deepstacked litter at a commercial dairy operation (Girisan dairy-livestock cooperatives). Eleven samples were obtained from experimentally processed BL representing ensiled, deepstacked and composted litter (Kwak et al., 2004).

For the hygienic safety experiment of long-term stored BL pellets, 20 kg lots each of ensiled, deepstacked and composted BL were pelletized using pelletizing equipment manufactured by Daegu-Sanggongsa of Korea. Ensiled and deepstacked BL were pelletized with and without 5% molasses. The diameter of pelletizing die was 8 mm. Each of the pelletized products was stored in a clean brown paper container with the upper side open for ventilation. Twenty kg lots of pelletized, ensiled (with and without 5% molasses), deepstacked (with and without 5% molasses) and composted BL were stored at ambient temperature for 100 days. Samples were taken from the center of the containers and analyzed for total coliform, pathogenic bacteria and mycotoxins.

Exp. 2: Hygienic safety of beef from cattle fed processed BL

Holstein bulls (avg BW 290 kg) were randomly assigned five to the control group and five to the BL-fed group. During a 12 week growing period, the control group was fed 7.9 kg of formulated mix and 1.8 kg of rice straw (as-fed basis) daily per head. The BL-fed group received 6.5 kg of formulated mix, 1.4 kg of BL (replacing 1.4 kg of formulated mix) and 2.2 kg of rice straw. All diets were supplied to satisfy the nutrient requirement of growing Holstein bulls (NRC, 2000). BL was blended and fed with formulated mix to prevent a possible palatability problem of BL. Then during the next 9 weeks of an early fattening period, the control group was fed 10 kg of formulated mix and 2 kg of rice straw, and the BL-fed group received the same as the control group with 1 kg of BL added. During the next 8 weeks of late fattening period, the amount of BL in the diet was reduced from 1 to 0.5 kg. Because the energy level of BL is relatively low, its provision was reduced stepwise during the fattening period. Daily feeding time was 0700 and 1800 h. Rice straw was supplied *ad libitum* during the whole experimental period. Water was available to the animals at all times. Health of animals was observed and recorded on the daily basis. The chemical composition of BL fed was as follows: dry matter 90.6%, organic matter 88.0%, crude protein 12.4%, ether extract 0.8%, and acid detergent fiber 48.5%. For BL-fed animals, feeding BL was withdrawn for 14 days before slaughtering.

All the animals were slaughtered at the Hannaeng Animal Processing Plant. Each of approximately 2 kg of *longissimus* muscle samples was taken from the carcass, frozen for 48 h and used for further analysis.

Microbial and chemical analyses

All the samples of feed and meat were analyzed according to methods of AOAC (1990) and Foodcode (2003) as follows. Total bacterial count by the standard plate method and total coliform count were analyzed quantitatively. Pathogenic bacteria such as *E. coli, E. coli O157:H7, Salmonella, Proteus* and *Listeria* were qualitatively analyzed.

Heavy metals including Cu, Fe, Zn, Mn, As, Pb and Cd were analyzed by Atomic Absorption Spectrophotometer (SpectraAA-300A, Varian Techtron, USA), and Hg by Mercury Analyzer (Rigaku SP-3A, Nippon Inc., Japan). For mycotoxins, aflatoxin B_1, B_2, G_1 and G_2 were qualitatively and quantitatively analyzed by High Performance Liquid Chromatography (Waters 600E, Waters Inc., USA). Detection sensitivities were in the range of 0.5-50 ppb depending upon kinds of metals and their recovery rates were over 90%.

Medicinal drug residues analyzed included virginiamycin, zinc bacitracin, chlortetracycline, tylosin, penicillin, enramycin, neomycin-H_2SO_4, chloroxytetracycline, oxytetracycline, salinomycin, sulfa substances (sulfamonomethoxine, sulfadimethoxine, sulfamethazine, sulfamerazine, and sulfaquinoxaline), and the coccidiostats (nicarbazin and amprolium). The antibiotics were detected by bioassay using standard strains of bacteria: *Bacillus cereus* var. *mycoides* ATCC 11778, *Micrococcus luteus* ATCC 9341, *Bacillus stearothermophilus* var. *B. calidolactis* C-953, *Pseudomonas syringae* ATCC 12885, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Micrococcus luteus* ATCC 10240, and *Corynebacterium xerosis* NCTC 9755. Samples which tested positive for an antibiotic were further analyzed quantitatively. Detection sensitivities of antibiotics were in the range of 0.01-0.05 ppm and their recovery rates were in the range of 65-92%.

Pesticide residues analyzed were as follows; BHC, heptachlor, aldrin, captan, endosulfan sulfate, dieldrin, endrin, captafol, tetradifon, cyhalothrin, permethrin, penconazole, cypermethrin, fenvalerate, tralomethrin, chlorothalonil, dicofol, folpet, DDD, DDT, bifenthrin, cyfluthrin, flucythrinate, fluvalinate, and deltamethrin for organic chlorine substances; methamidophos, acephate, ethoprophos, terbufos, etrimfos, chlorpyrifos-methyl, fenthion, chlorfenvinphos, vamidothion, carbophenothion, fensulfothion, phosmet, phorate, chlorpyrifos, parathion-methyl, fenitrothion, phenthoate, fenamiphos, ethion, edifenphos, dichlorvos, diazinon, omethoate, dimethoate, pirimiphos-methyl, malathion, parathion, isofenphos, methidathion, and EPN for phosphorus substances; oxamyl, methomyl, aldicarb, propoxur, carbofuran, carbaryl, ethiofencarb, bendiocarb, and methiocarb for carbamate substances; ethylene dibromide substances; thiabendazole substances. Substances of organic chlorine, phosphorus, and ethylene dibromide were analyzed on Gas Chromatography (Varian star 3600cx, Varian Techtron, USA) and substances of carbamate and thiabendazole on High Performance Liquid Chromatography (Termo Separation Products, USA). Detection sensitivities were in the range of 0.1-0.5 ppb for organic chlorine substances and in the range of 1-10

Table 1. Change in bacterial presence according to deepstacking of broiler litter in each of 3 trials[1, 2]

Deepstacking	Total bacteria	Total coliform	*E. coli*				
	---------- $\log_{10}$ cfu/g[3] ----------		*E. coli*	*E coli O157:H7*	*Salmonella*	*Listeria*	*Proteus*
Trial 1							
Pre	8.637[a]	4.177[a]	-	-	-	-	+
Post	5.368[b]	0[b]	-	-	-	-	-
SE	0.457	0.376					
Trial 2							
Pre	9.382[a]	6.419[a]	+	-	+	+	+
Post	6.471[b]	0.189[b]	-	-	-	-	-
SE	0.658	0.369					
Trial 3							
Pre	9.699[a]	6.135[a]	+	-	+	+	+
Post	5.181[b]	0[b]	-	-	-	-	-
SE	0.752	0.412					
Trial 1-3							
Pre	9.240[a]	5.577[a]	+	-	+	+	+
Post	5.673[b]	0.063[b]	-	-	-	-	-
SE	0.366	0.223					

[1] Means of 3 observations for each trial. [2] '+' is a positive response and '-' is a negative response.
[3] Colony-forming unit per gram of wet sample. [a, b] Means with different superscripts within the same column differ ($p<0.05$).

Table 2. Heavy metals and aflatoxin residues in deepstacked, ensiled and composted broiler litter and comparisons with the maximum tolerant level[1]

Item	Range	Mean	Max. tolerant level	
			KMAF[2]	NRC[3]
Heavy metals				
Cu, ppm	54-235	148	-	100
Fe, ppm	153-3,985	940	-	-
Mn, ppm	225-453	336	-	1,000
Zn, ppm	166-605	274	-	500
As, ppm	0.1-2.0	0.6	100	50
Pb, ppm	ND-9.2	2.4	20	30
Cd, ppm	ND-1.4	0.7	2.5	0.5
Hg, ppb	3.4-30.6	10.7	500	2,000
Aflatoxin[4]				
B_1, ppb	ND-0.57	0.08	50	20[5]
B_2, ppb	ND	ND		
G_1, ppb	ND	ND		
G_2, ppb	ND	ND		

[1] Sample number=16. [2] In feed ingredients (KMAF, 2001).
[3] Cu, Mn, Zn and As for cattle and Pb, Cd and Hg for human food (NRC, 1980).
[4] Value in the maximum tolerant level is a sum of B_1, B_2, G_1 and G_2.
[5] In feed ingredients (FDA, 1987).

ppb for phosphorus, carbamate and ethylene dibromide substances. Their recovery rates were in the range of 75-114% depending upon the individual pesticide analyzed.

Statistical analysis

Data obtained were analyzed using General Linear Model (SAS Institute, Inc., 1990). Mean comparisons of before and after deepstacking in Exp. 1 and between the control and BL-fed groups in Exp. 2 were made with studentized-t test (SAS Institute, Inc., 1990).

RESULTS AND DISCUSSION

Exp. 1. Hygienic safety of processed BL

Pathogenic microorganisms : The total bacterial count for unprocessed BL for the three trials was in the level of 10^8-10^9 cfu/g, and the total coliform count, in the level of 10^4-10^6 cfu/g (Table 1). Among the pathogenic bacteria, *Proteus* was detected in all three trials. *E. coli*, *Salmonella*, and *Listeria* were detected in two trials, and *E. coli O157:H7* was not detected in any of the three trials.

After deepstacking, the total bacterial count for all three trials decreased to 10^4-10^6 cfu/g. Therefore, the deepstacking process reduced the total bacterial count by a factor of approximately 1,000. The deepstacking process eliminated the total coliform bacteria in BL used in trials 1 and 3, and in trial 2 they were detected in one of three samples at the level of 3 cfu/g. However, since this value is lower than the 10 cfu/ml tolerance for milk (Foodcode, 2003), on this basis the BL was considered hygienically safe. *E. coli*, *Salmonella*, *Listeria* and *Proteus* were detected in unprocessed BL, and after the litter was deepstacked they were all eliminated. *E. coli O157:H7* was not detected either before or after deepstacking. In the state of Georgia, US, *E. coli O157:H7* was not detected in raw BL and no pathogenic microorganisms were detected after the BL was deepstacked (Martin et al., 1998). *Salmonella*, *Shigella* and *Proteus* were present in unprocessed BL, however, they were not detected after the litter was deepstacked (Chaudhry et al., 1996). These two reports support the results of our study. The rapid reduction of total bacterial count and the elimination of pathogenic microorganisms by deepstacking could be due to spontaneous heating and ammonia generation that occur during deepstacking of BL (Ruffin and McCaskey, 1990). Lu et al. (2003) reported that various aerobic bacteria which are known to actively decompose organic matter during the deepstacking could antagonistically suppress the growth of pathogenic microorganisms. The generation of heat, ammonia and competitive inhibition by non-pathogenic bacteria during the deepstacking process must eliminate pathogenic bacteria from litter.

Heavy metals and mycotoxins : Heavy metals in processed BL and their maximum permitted tolerance in animal feeds are presented in Table 2. Compared with conventional feedstuffs, such as maize and soybean (KMAF, 2002), BL has much higher levels of heavy metals. Among them, Cu has been a major concern, because Cu toxicity has been reported to occur in sheep fed BL in US (Fontenot et al., 1971). Generally, Cu is added to the broiler poultry diet to promote their growth and to increase feed efficiency. In this study, the average level of Cu in BL was 148 ppm, which is much lower than the 255 ppm level reported in Alabama (McCaskey and Anthony, 1979) and the 593 ppm level reported in Virginia, US, (Westing et al., 1985). Sheep are sensitive to high Cu levels in their diets; however, cattle have higher tolerance to dietary Cu (Webb et al., 1974). In this study, Fe levels in the BL varied considerably, which might be due to soil contamination of the litter when the litter was removed from the dirt floor broiler houses. Levels of Fe, Mn, Zn and Cu in BL were less than half of those reported in the state of North Carolina, US (Hopkins and Poore, 2001). Since BL is more frequently removed from broiler houses in Korea, the bedding material makes up a larger fraction of the BL than broiler excreta which contains the major portion of the minerals in BL. The second possible explanation is the quantity of mineral premix added to broiler poultry diets in Korea is relatively low.

Among toxic heavy metals, levels of As, Pb and Hg in processed rice hulls-bedded BL were much lower than the tolerances stipulated by the governments of Korea (KMAF, 2001) and US (NRC, 1980). The average level of Cd slightly exceeded the tolerance for food presented in US, but was just quarter of that in Korea. Westing et al. (1985)

Table 3. Medicinal drug residues in deepstacked, ensiled and composted broiler litter[1, 2]

Item	Range (ppm)	Mean (ppm)	Sample No. detected per 16 samples
Salinomycin	0.1-0.2	0.13	16
Nicarbazin	0.01-0.07	0.03	16
Amprolium	0.1-0.7	0.41	16
Sulfa substances[3]	ND-1.1	0.21	11
Chloroxytetracycline	ND-0.004	0.0003	1
Oxytetracycline	ND-0.001	0.00006	1
Virginiamycin	ND	ND	0
Zinc Bacitracin	ND	ND	0
Chlortetracycline	ND	ND	0
Tylosin	ND	ND	0
Penicillin	ND	ND	0
Enramycin	ND	ND	0
Neomycin-H_2SO_4	ND	ND	0

[1] Sample number=16.
[2] 'ND' is non-detected and positively responsed samples were analyzed quantitatively.
[3] Sulfa substances included sulfamonomethoxine, sulfadimethoxine, sulfamethazine, sulfamerazine, and sulfaquinoxaline.

Table 4. Pesticide residues (ppb) in deepstacked, ensiled and composted broiler litter[1]

Substance	Content[2]	Sample No. detected per 16 samples	Max. tolerant level[3]
Organic chlorine			
BHC	8, 12, 108	3	200
Dieldrin	0.4, 3	2	20
Endosulfan sulfate	5, 10	2	-
Cyfluthrin	50	1	-
Others[4]	0	0	-
Phosphorus			
Isofenphos	70-530	9	-
Parathion	4-70	5	1,000
Methidathion	250	1	-
Etrimfos	990	1	-
Chlorpyrifos-methyl	136	1	6,000
Fenitrothion	34	1	6,000
Others[4]	0	0	-
Carbamate			
Carbofuran	180-830	10	-
Others[4]	0	0	-
Ethylene dibromide	all ND	0	500
Thiabendazole	all ND	0	5,000

[1] Sample number=16.
[2] ND: non-detected and positively responded samples were analyzed quantitatively.
[3] Notice 2001-61 by Korean Ministry of Agriculture and Forest for formulated mix (KMAF, 2001).
[4] Other items analyzed, but not presented here in detail were all negative.

reported that the level of toxic heavy metals in BL used in US beef cattle production systems was hygienically safe. Based on these reports, the levels of heavy metals in processed rice hulls-bedded BL are considered to be safe.

For mycotoxin, aflatoxin was analyzed as a typical mycotoxin in processed BL and presented in Table 2. It was not detected in 13 of 16 samples, but aflatoxin B_1 out of B_1, B_2, G_1, G_2 was detected in 3 samples at the level of 0.12 - 0.57 ppb. This level is much lower than the 50 ppb tolerance for by-product feed stipulated in the feed control regulation of Korea (KMAF, 2001) and the 20 ppb level reported for the US (FDA, 1987). In our study, white fungi were observed more frequently on the surface of BL treated with 5% molasses than without molasses. Thus, the nutrients of molasses seemed to stimulate the fungal growth. Therefore, it was anticipated that the addition of molasses would adversely affect the long term storage of BL.

Mycotoxin is generally associated with maize, peanut, and oil meals. Lee (2003) reported that maize and soybean meal imported into Korea were contaminated with aflatoxin at the level of 0.1-7 ppb for maize and 1.4-20 ppb for soybean meal. Russell et al. (1991) reported that 20% of the samples collected from seven commercial feed mills in the Midwestern states, US were contaminated with aflatoxin during 1988-1989. The concentration of aflatoxin in contaminated maize and cottonseed was over 300 ppb, causing a serious hygienic problem (Price et al., 1993). Thus, the mycotoxin contamination of commercial feed grade maize appeared to be a more serious problem than with BL. Broiler litter has a pH of about 8, and intrinsically high levels of ammonia which is inhibitory to the growth of most molds. Ammonia generated by the decomposition of BL protein during the deepstacking of BL is toxic to fungi (Ruffin and McCaskey, 1990; Davis, 2002). The high pH and ammonia content of BL must prevent the growth of molds that produce mycotoxins.

Medicinal drug residues : Medicinal drugs, such as antibiotics and other antibacterial substances, are fed to broilers and excreted in the poultry feces. The presence of 13 major antibiotic and antibacterial substance residues in processed BL is presented in Table 3. Salinomycin, nicarbazin, and amprolium were detected in all 16 samples and sulfa substances, in 11 of 16 samples. Chloroxytetracycline and oxytetracycline were detected in only 1 sample. Virginiamycin, zinc bacitracin, chlortetracycline, tylosin, penicillin, enramycin and neomycin-H_2SO_4 were not detected in any of the samples.

It is conceivable that some antibiotics and antibacterial substances should be present in BL, but the actual concentrations of residues found in BL at the ppb level were much lower than expected. It appeared that medicinal drugs added to the broiler diet normally in concentrations of tens or hundreds of ppm were diluted to the level of ppb in processed BL. This could be due to dilution of the drugs by mixing excreta with bedding material and some of the drugs might have been altered by microbes which can make their

Table 5. Bacterial presence in deepstacked, ensiled and composted broiler litter pellets after 100 days of storage[1,2]

Item	Total coliform (cfu/g)[3]	*E. coli*	*E. coli O157:H7*	*Salmonella*	*Listeria*	*Proteus*	Aflatoxin (ppb)		
							B_1	B_2	G_2
Range	ND-37[4]	-	-	-	-	-	ND-2.7	ND-3.8	ND-0.1
Mean	10.6	-	-	-	-	-	0.7	0.8	0.02
SD	16.3	-	-	-	-	-	1.1	1.7	0.04
Max. tolerant level[5]		-	-	-	-	-	Sum of B and G=50		

[1] Sample number=5. [2] ND: non-detected, '-' is a negative response and '+' is a positive response.
[3] Colony-forming unit per gram of wet samples.
[4] Non-detected for 3 molasses-untreated BL pellets samples and detected positively for 2 molasses-treated BL pellets samples.
[5] In feed ingredients (KMAF, 2001).

detection difficult. Caswell et al. (1977) reported that the antibiotic substances remaining in BL could be decomposed by fermentative microorganisms during fermentation.

To ensure safety from medicinal drug residues in meat of animals fed BL, a litter feeding withdrawal period should be used for animals destined for slaughter. This procedure should be done also for animals on commercial feed diets that are administered drugs which require a withdrawal period before slaughter. Most of the medicinal drugs that are permitted in the diet of poultry are also approved for beef cattle. To ensure that there are no illegal levels of drug residues in the tissues of beef cattle destined for slaughter, litter should be withdrawn from the animal diets for at least 15 days prior to slaughter.

Pesticide residues : In Table 4 are presented the concentrations of pesticides in BL and the maximum tolerance of the pesticides permitted in commercial animal feeds in Korea. The pesticides analyzed included substances of organic chlorine (30 types), phosphorus (30 types), carbamate (9 types), all of ethylene dibromide and all of thiabendazole. Among the 30 types of analyzed organic chlorine, only 4 types were detected in 8 samples and these types, namely, BHC, dieldrin, endosulfan sulfate and cyfluthrin were detected in 1-3 samples of all 16 samples at the ppb level. Among the 30 types of analyzed phosphorus substances, 6 types were detected, and isofenphos was detected most frequently, followed by parathion. Methidathion, etrimfos, chlorpyrifos-methyl and fenitrothion were detected in only one sample. Among the 9 analyzed carbamate substances, only carbofuran was most frequently detected in relatively high concentration. Ethylene dibromide and thiabendazole substances were not detected at all.

When compared with the tolerance of 7 types specified in KMAF (2001) which were directly comparable on the basis of feed control regulation in Korea, the levels of pesticide residues in processed BL were extremely low. Diazinon, fenthion, carbaryl, malathion, phenthoate, pirimiphos-methyl, dichlorvos, DDT, endrin and heptachlor presented in the tolerance regulation were not detected in BL at all. For a reference, pesticides (not presented in Table 4) undetected in all the 16 BL samples were DDD, DDT, BHC, heptachlor, aldrin, captan, endrin, endosulfan sulfate, captafol, tetradifon, cyhalothrin, permethrin, cypermethrin, fenvalerate, tralomethrin, chlorothalonil, dicofol, folpet, bifenthrin, flucythrinate, fluvalinate, and deltamethrin for organic chlorine substances, methamidophos, acephate, ethoprophos, terbufos, fenthion, chlorfenvinphos, vamidothion, carbophenothion, fensulfothion, phosmet, phorate, chlorpyrifos, parathion-methyl, phenthoate, fenamiphos, ethion, edifenphos, dichlorvos, diazinon, omethoate, dimethoate, pirimiphos-methyl, and malathion for phosphorus substances, and oxamyl, methomyl, aldicarb, propoxur, carbaryl, ethiofencarb, bendiocarb, and methiocarb for carbamate substances.

It was also reported that contents of pesticide residues in dehydrated caged layer waste commercially available in California, US was also very low at the ppb level (Hamblin, 1980). While the tolerance of DDT residue in cereals is 2.0 ppm (Bhattacharya and Taylor, 1975), DDT and its analogs were not detected in processed BL at all. With regard to pesticide residues, conventional cereals for feed seemed to arouse more concern than BL.

In conclusion, pesticide residues detected in processed rice hulls-bedded BL was hygienically safe. Also, there has been no report as yet, stating that pesticide residues caused any hygienic problems.

Safety of BL pellets stored for a long term : BL was processed by various methods, then pelletized and stored at ambient temperature for 100 days. After storage the pelletized litter was analyzed for total bacterial count and for specific pathogenic bacteria. In palletized BL, total coliform and pathogenic bacteria, such as *E. coli*, *E. coli O157:H7*, *Salmonella*, *Listeria* and *Proteus*, were not detected (Table 5). For ensiled or deepstacked BL treated with 5% molasses and pelletized, total coliform counts ranged from 16 to 37 cfu/g and no pathogenic bacteria were detected. The total coliform count increased markedly for palletized BL stored for 100 days.

For mycotoxin, the sum of aflatoxin B_1, B_2 and G_2 contents was in the range of 0.1-3.8 ppb in each of five samples (Table 5). These values were much lower than the 50 ppb tolerance for by-product feed (KMAF, 2001). Especially, as molasses was added while BL was pelletized,

Table 6. Pathogenic bacterial presence in *longissimus* muscle of beef cattle fed different diets[1, 2]

Item	Diet	
	Control	Broiler litter[3]
E. coli	-	-
E. coli O157:H7	-	-
Salmonella	-	-
Listeria	-	-
Proteus	-	-

[1] n=5 observations.
[2] '-' is a negative response and '+' is a positive response.
[3] Broiler litter was processed by deepstacking.

Table 7. Heavy metal residues in *longissimus* muscle of beef cattle fed different diets[1, 2]

Item	Diet		SE
	Control	Broiler litter[3]	
Zn, ppm	24.69	30.54	3.07
Fe, ppm	12.33	14.21	2.85
Mn, ppm	0.15	0.02	0.07
Cu, ppm	0.99	0.57	0.22
Cd, ppm	0.02[a]	ND[b]	0.01
Pb, ppm	ND	ND	-
As, ppb	3.62	2.91	1.34
Hg, ppb	0.25	0.20	0.13

[1] Means of 5 observations. [2] ND: non-detected.
[3] Broiler litter was processed by deepstacking.
[a, b] Means with different superscripts within the same row differ ($p<0.05$).

more white fungi were observed on the surface of BL pellets after 100 days of long term storage.

In conclusion, BL pellets without molasses treatment were safe from pathogenic bacteria or mycotoxin notwithstanding long term storage for 100 days. However, if molasses was added and the litter pelletized, there was a risk of coliform contamination.

Exp. 2. Hygienic safety of meat

Pathogenic microorganisms : The *longissimus* muscles collected after slaughtering Holstein bulls fed BL were analyzed for pathogenic microorganisms (Table 6). Pathogens, such as *E. coli*, *E. coli O157:H7*, *Salmonella*, *Listeria*, and *Proteus*, were not detected in meat of the control group or the BL-fed group of animals. These results indicate that the feeding of BL to beef animals likely does not increase the risk of pathogen contamination of meat compared to animals fed conventional diets.

Heavy metals : For heavy metal residues in the rib muscle (Table 7), there was no difference in levels of Cu, Fe, Mn, and Zn between control group and BL-fed group of animals ($p>0.05$). Studies have shown that diets with excessive Cu levels cause the dietary Cu to accumulate in the liver and not in the muscle tissue (Westing et al., 1985). In the case of toxic heavy metals, Cd detected in the muscle of the control group of animals was not detected in the BL-fed group ($p<0.05$). Probably, the Cd remaining in the BL at the average value of 0.7 ppm in Exp. 1 might not be accumulated in the rib muscle after the proper withdrawal prior to slaughtering. Pb was not detected in the muscle of control group nor in the BL-fed group of animals. There was almost no difference in levels of muscle As and Hg between control group and BL-fed group of animals ($p>0.05$). When compared with the tolerance of heavy metals for general food stipulated by Foodcode (2003) (Cd 0.2 ppm, Pb 2 ppm and Hg 500 ppb), the values of these metals in this study were very low. In a similar experiment in Virginia, US, Westing et al. (1985) fed maize-BL silage to beef cattle for 200 days, slaughtered the animals after one day of withdrawal, and assayed heavy metals in the rib muscle. Compared to the results of Westing et al. (1985), the As level was similar, but levels of Cu, Fe, Mn, Zn, Cd, Pb and Hg were much lower in our study. This dissimilarity in the two studies was apparently due to the difference in the original level of heavy metals in BL, BL feeding quantity, BL feeding term and(or) withdrawal period.

Table 8. Drug residues in *longissimus* muscle of beef cattle fed different diets[1, 2]

Item	Diet	
	Control	Broiler litter[3]
Salinomycin	ND	ND
Nicarbazin	ND	ND
Amprolium	ND	ND
Sulfa substances[4]	ND	ND
Chloroxytetracycline	ND	ND
Oxytetracycline	ND	ND

[1] n=5 observations. [2] ND:non-detected.
[3] Broiler litter was processed by deepstacking.
[4] Sulfa substances included sulfamonomethoxine, sulfadimethoxine, sulfamethazine, sulfamerazine, and sulfaquinoxaline.

Medicinal drug residues : There were no residues of medicinal drugs detected in the rib muscle for both the control and the BL-fed groups of animals (Table 8). The six drug residues detected in processed BL of Exp. 1 were not detected in the rib muscle of the animals fed BL. As presented in Table 3, all of the medicinal drugs not detected in processed BL also were not detected in the rib muscle (data, not presented). In Korea, the tolerance in beef of some medicinal drugs listed in Table 8 was stipulated in the range of 0.1-0.5 ppm, except for salinomycin which has a zero tolerance (Foodcode, 2003).

In conclusion, it was determined that 14-day withdrawal period from the feeding of deepstacked rice hulls-bedded BL to beef cattle before slaughtering makes the beef safe without any residue of medicinal drugs.

Pesticide residues : Seventy one pesticide residues were analyzed in the rib muscle of animals fed control and BL diets (data, not presented). 4 residues were detected in 5 samples for the control group, namely, DDT 19 ppb, penconazole 45 ppb, endosulfan sulfate 12 ppb and captan 65 ppb. In BL-fed group, 3 residues were detected in 5 samples, namely, DDT 30 ppb, chlorpyrifos-methyl 0.5 ppb

and aldrin 3 ppb. These values were much lower than the tolerance of pesticides for beef presented in the Foodcode (2003), such as DDT 5.0 ppm, chlorpyrifos 2.0 ppm, aldrin 0.2 ppm, and endosulfan sulfate 0.1 ppm. The pesticide residues detected in the rib muscle of the BL-fed cattle were not the same as those detected in BL of Exp. 1 except chlorpyrifos-methyl. Thus it seemed that some of the residues detected in meat had originated from feeds other than the BL. With regard to pesticide residue in tissues of animals fed BL, meat of cattle fed BL did not appear to pose any more pesticide residues hazard than the meat of animals fed the conventional diet.

Overall, these results showed that meat of cattle fed processed rice hulls-bedded BL was hygienically safe from pathogenic bacteria, heavy metals, medicinal drugs and pesticide residues.

IMPLICATION

In some regions of the world, as in Korea, where rice hulls-bedded BL is frequently (more than three times annually) removed from the broiler poultry houses, the relatively low component of broiler excreta in BL may have an advantage of diluting the heavy metals and medicinal drug residues concentrations remaining in the BL. The low Cu level of BL in Korea may have another advantage of preventing Cu toxicity because the excess Cu plays a role as a limiting factor in the ruminant diet. Problems of pesticide residues and mycotoxin were less serious in BL than in conventional feedstuffs. BL pellets manufactured for ease of handling and transportation could be stored for up to 100 days without evidence of pathogenic bacteria and mold growth. Longer term storage of pelletized BL compared to pelletized conventional feed was apparently due to the antimicrobial effect of the ammonia component of BL. In conclusion, use as feed of cheap and hygienically safe rice hulls-bedded BL will make possible not only considerable feed cost reduction for the beef farmers but also less expensive and safe beef supply to the consumers.

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