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A Nutritional Evaluation on Whole Cottonseed Removed Germination Ability by Heat-treatment

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ABSTRACT

In Korea, wide spread use of whole cottonseed, which is primarily a GMO plant imported from foreign countries and being fed to animals as raw state, has aroused concern that it may disturb the existing ecology of the country unless dispersion of the seed is under proper control. The objective of this study was to elucidate the changes in various nutritive parameters due to heat treatment and to determine the effective condition for removing germination ability of whole cottonseed (WCS). Of the various temperatures applied (76, 78, 80, 85, 100°C/30 min) 85°C for 30 min was confirmed to be the lowest temperature treatment which resulted in a complete removal of the germination ability of WCS. Therefore, based on the determined temperature condition (85°C 30 min) we tried to examine the changes of various nutritional parameters, including nutrient composition, *in vitro* digestibilities and ruminal protein degradabilities, comparing raw whole cotton seed (RWCS) and heated whole cotton seed (HWCS). Some changes in amino acid composition were observed with heat treatment of WCS, but these were regarded to originate from the variation in plant quality and seed morphology, which are usually affected by different environmental factors during the vegetation period. As for fatty acid composition, no significant differences were observed to occur during heat treatment. However, WCS heated at 85°C for 30 min in a circulating oven showed a significant decrease ($p<0.05$) of *in situ* rumen degradability in both dry matter (DM) and crude protein (CP), as compared to raw WCS. Overall results obtained in the study indicate that the heating condition used in this study, which was proven to be the most appropriate and economic to remove germination ability of WCS, may also improve the nutritional value of the ruminant with regard to reducing its protein degradability within the rumen.
(**Key words :** Whole cottonseed, Germination ability, Heat treatment, Degradability)

Ⅰ. INTRODUCTION

Whole cottonseed (WCS) is a popular feedstuff used for dairy cows due to its high fiber, high energy and high protein content (Bernard et al., 1999). In a nationwide survey to determine the feedstuffs fed to lactating dairy cows, it was reported that approximately 40% of dairy producers in the United States fed WCS (Mowrey and Spain, 1999). WCS is one of the major component feeds used for TMR in Korea. Recently genetic modifications have produced cotton plants more resistant to pests and tolerant to herbicides (Bertrand et al., 2005). On the other side, like Korean circumstances, wide spread use of whole cottonseed, which is mostly genetically modified organism (GMO) plant imported from foreign countries and being fed to animals as raw state, arouses worries that it may disturb existing ecology of the country unless dispersion of the seed is under proper control. Even a piece of the seed within the excreta does not lose its ability of germination, which would occasionally come in sight on the farmstead.

One of the potential processing methods to remove germination ability of the seed would be heat treatment. Generally accepted heating temperature to get rid of germination ability for hard seed plant species is presumably about more than 70℃, since Beena and Jayaram (2010) found that the seeds of soybean and green pea withstood high temperature up to 70℃ for 10 days even if high temperature treatment reduced the rate of germination.

On the other hand, heat treatment on the feed has long been an important processing method to improve its

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nutritive values; for instance, heat processing is the most used treatment in North America to decrease microbial degradability of feed protein in the ruminant (National Research Council, 2001). Various heat treatments have been used to decrease the solubility of crude protein and increase the rumen undegradable protein (RUP) fraction of different feedstuffs by blocking reactive sites for microbial proteolytic enzymes (Mabjeesh et al., 2000). WCS is relatively high in N, being used to supply an important portion of the dietary N required by dairy cows (Pires et al., 1997). Several studies on various protein sources have shown a correlation between increased milk production and decreased ruminal degradation of protein (Faldet and Satter, 1991; Sahlu et al., 1984). Heat treatment of WCS increased the amount of ruminally undegradable protein (Pena et al., 1986), however, the effect seemed highly dependent upon the level of heat input (Pires et al., 1997).

The study was therefore conducted to elucidate the effects of heat treatment on changes in various nutritive parameters under effective condition for removing germination ability of WCS.

Ⅱ. MATERIALS AND METHODS

The experiment was conducted after the protocol was approved by Institutional Animal Care and Use Committee (No. KUIACUC 20110511-2).

1. Treatment of whole cottonseed

Linted whole cottonseed (WCS) was purchased from KOREA SILO, Co., Ltd (Incheon, Korea), and heated using mechanical circulation oven (VS-1202D9, Vision Scientific Co., Bucheon, Korea) at 76, 78, 80, 85, 100℃ for 30min, respectively.

2. Chemical analysis

Shortly after drying both raw seeds (RWCS) and heated ones (HWCS) were ground in 2 steps, firstly using a disc mill (McCoy Corporation, BM-D-100, TX, USA) and secondly a laboratory centrifuge mill (Shinmyung BT, SMBT 3000, Daejeon, Korea). The stepwise grinding procedure was a useful preventive of segregation between the seed and the lint, enabling us to obtain homogeneous samples for chemical analysis as well as *in vitro* trial for digestibility. Samples were analyzed for DM, CP and lipid by AOAC procedures (AOAC, 1995). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using an ANKOM200 Fiber Analyzer (ANKOM Technology, NY, USA). Fatty acid composition were examined by gas chromatography with flame ionization detection (GC-FID) using a HP-6890GC instrument (Hewlett-Packard, CA, USA) with a FID and a HP-FFAP column (30 m × 0.32 mm × 0.25 μm). The oven temperature conditions were as follows: the initial temperature of the column, 100℃, was held for 2min, after which the temperature was stepped up by 4℃ /min to 240℃, where it was held for 20 min. The flow rate of the carrier gas (helium) flow rate was 1.5 mL/min. Fatty acids were identified by comparing the relative retention times of FAME peaks with those of standards. Total amino acid composition was determined with an amino acid analyzer (Biochrom 20, Pharmacia Biotech, Buckinghamshire, England). Samples were hydrolyzed in 6N HCl in evacuated sealed tubes at 110℃ for 24 h. The hydrolysates were evaporated to dryness in a vacuum evaporator and then diluted with sodium citrate buffer for analyzing amino acid.

3. Germination test

After sterilization of whole cotton seeds according to the procedure of Sato et al. (2005), three replicates of 30 seeds each of control and heat-treated were placed in sterilized Petri dishes and covered with lid plates which also lined with moistened filter paper. Petri dishes were watered as required to replace evaporation losses.

$$\text{Germination (\%)} = \frac{\text{No. of seeds germinated}}{\text{Total No. of seeds taken for germination studies}} \times 100$$

4. *In vitro* digestibility

Fresh rumen content from two ruminally-cannulated Holstein cows was collected 3h after morning feeding for use in the cultures. Approximately 1.5 L rumen fluid was

collected by straining the digesta through four layers of cheesecloth into a flask flushed previously with CO_2. In this trial, 0.5 g of each sample was placed in to 50 mL tubes, the incubation inoculum was prepared by diluting the inoculum with McDougall's buffer (McDougall, 1947) and fresh rumen liquor in a 1:4 ($v \cdot v^{-1}$) ratio and stirring in a water bath at 39°C with purging CO_2 until its use (10-15 min later). The inoculated test tubes were allocated in shaking incubator at 39℃ for 48 h. After incubation, the tubes were kept in ice and centrifuged at 10,000 rpm for 15 min and the supernatant was discarded. Samples were subjected to either a 48 h pepsin-HCl digestion as described by Tilley and Terry (1963). The residue remaining after drying was used to calculate the *in vitro* dry matter digestibility.

5. *In situ* study

Two ruminally cannulated Holstein heifers were used to determine *in situ* dry matter and protein degradability of both WCSs. The animals were maintained on a standard diet as TMR having a forage to concentrate ratio of 7 to 3.

WCS samples (3.5 g) were weighed in to 5 × 10 cm nitrogen-free polyester bags (ANKOM Technology, NY, USA) with a 50 μm mean pore size. Before placement in the rumen, the bags (n=24) were soaked briefly in water, and then introduced serially into the rumen and suspended for 2, 4, 8, 16, 24 and 48 h in four replicates for each incubation time. Solubility at 0 h was evaluated by immersing the bags in warm water (39℃). At the end of each incubation time, the bags were removed from the rumen and rinsed thoroughly with cold tab water until rinsing water became colorless. Bags then were placed in a forced air oven set at 60℃ for 48 h. The residue was analyzed for dry matter by drying oven (60℃/48 h), and nitrogen content by micro-Kjeldahl method.

6. Statistical analysis

Statistical significant differences of means at $p<0.05$ were calculated using one-way ANOVA indicated by different letters above bars.

Ⅲ. RESULTS AND DISCUSSION

When WCS were subjected to heat treatment at 76, 78 and 80℃ for 30 min, the germination rate was significantly decreased (Fig. 1). But complete elimination of germination ability was attained when we applied the temperature as high as 85℃ or 100℃ for the same duration. In the study of Beena Anto et al. (2010) could observed a reduction in the germination percentage of pea and soybean to 10% and 17%, respectively, when they increased the temperature up to 70℃. The heating level of 85℃ for 30 min used in this experiment may be economically highest one to attain a complete removal of germination ability for WCS. Drying at high temperature leads to significant reduction of moisture content and concomitant loss of viability in seeds (Meng et al., 2003). Therefore, based on the optimal temperature condition (85℃, 30 min) we tried to examine the changes of various nutritional parameters including nutrient composition, *in vitro* digestibilities and ruminal protein degradabilities, comparing between raw (RWCS) and heated (HWCS).

No significant differences were found in proximate analysis of nutrient contents between RWCS and HWCS ($p>0.05$) (Table 1). Fat (EE) contents were quite similar to those reported by Bertrand et al. (2005). Minor differences observed in the composition of whole cottonseed may be

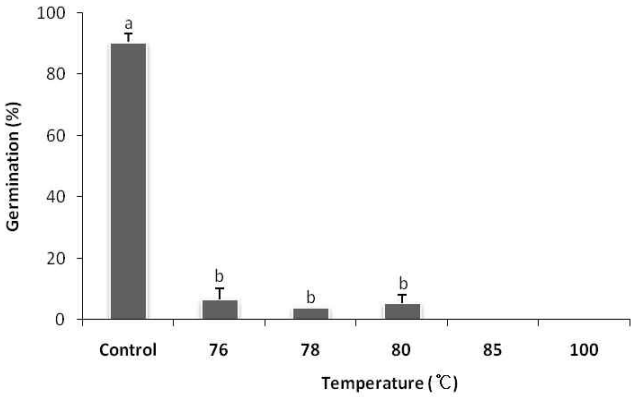

Fig. 1. Effect of heat treatment on germination of whole cottonseed (WCS).
WCS was heated by mechanical circulation oven at 76, 78, 80, 85, 100℃ for 30min, respectively, with intact one as Control.
a,b Values with different letters above each bar are significantly different ($p<0.05$) among different treatments.

Table 1. Chemical composition of raw whole cottonseed (RWCS) and heat treated whole cottonseed (HWCS)

(%, DM basis)

Item	RWCS	HWCS[1)]
DM	93.65±0.11[2)]	94.52±0.19
Ash	3.57±0.14	3.59±0.10
CP	22.21±0.07	20.76±0.06
EE	17.76±0.23	17.11±0.11
NDF	47.23±0.26	48.22±0.22
ADF	36.01±0.19	35.52±0.24
NFC[3)]	9.23±0.18	10.32±0.13

[1)] HWCS = heat-treated whole cottonseed at 85℃ for 30 min.
[2)] Values are mean ± standard deviation (n=3).
[3)] NFC=100－(Ash+CP+EE+NDF).
DM : dry matter, CP : crude protein, EE : ether extract, NDF : neutral detergent fiber, ADF : acid detergent fiber, NFC : non-fiber carbohydrate.

due in part to size of the seed, lint content of the hull and weather conditions (e.g. average rainfall and temperature). Table 2 shows a comparison of amino acid contents between RWCS and HWCS. Changes in some amino acid content were observed by heat treatment; i.e. higher levels of glutamic acid, glycine, histidine and threonine and lower levels of methionine and leucine were obtained in HWCS as compared to RWCS ($p<0.05$). Other amino acids existed in similar contents for both kinds. The differences, however, seemed not to be associated with heat treatment but the partly to reflect some variation originated from different sampling lot and also the variation that might come from different environmental factors affecting plant quality and seed morphology during vegetation period (Van Soest, 1994). As for fatty acid composition, no significant differences were observed by the heat treatment (Table 3). Linoleic acid ($C_{18:2}$) was consistently present in the highest quantity averaged 56% of the total fatty acid. The fatty acid in next highest quantity was palmitic acid ($C_{16:0}$) showing 23% on

Table 2. Amino acid contents of raw whole cottonseed (RWCS) and heat-treated whole cottonseed (HWCS)

(mg/100g)

Components	WCS	HWCS[1)]
Aspartic acid	1,959.2±11.9[2)]	2,013.3±26.42*
Serine	888.3± 3.5	909.2± 5.1
Glutamic acid	4,587.4± 4.3	4,700.6± 7.7*
Glycine	861.3± 5.7	874.4± 8.9*
Histidine	589.0± 2.8	599.4± 2.5*
Threonine	635.7± 4.5	652.9± 5.4*
Arginine	2,430.6±10.7	2,460.8±32.3
Alanine	866.9± 7.5	877.7±12.2
Proline	779.2± 7.8	792.6±25.8
Tyrosine	494.9± 3.3	467.0±40.0
Valine	960.4±29.5	973.7±17.8
Methionine	255.8± 2.2	245.8± 2.6*
Lysine	969.7± 4.9	992.5±23.0
Isoleucine	706.1± 8.1	705.3±11.1
Leucine	1,331.0±23.4	1,294.7±10.2*
Phenylalanine	1,196.0±12.1	1,205.1±19.2

[1)] HWCS = heat-treated whole cottonseed at 85℃ for 30 min.
[2)] Values are Mean ± standard deviation (n=3).
* indicates significant differences at $p<0.05$ as calculated with Student's T-test.

Table 3. Fatty acid composition of raw whole cottonseed (RWCS) and heat-treated whole cottonseed (HWCS)

Fatty acid	(% of total fat)	
	RWCS	HWCS[1)]
Myristic acid ($C_{14:0}$)	0.7	0.7
Palmitic acid ($C_{16:0}$)	23.0	23.0
Palmitoleic acid ($C_{16:1}$)	0.5	0.5
Stearic acid ($C_{18:0}$)	2.6	2.7
Oleic acid ($C_{18:1}$)	16.0	16.2
Linoleic acid ($C_{18:2}$)	56.5	56.3
Linolenic acid ($C_{18:3}$)	0.2	0.2
Arachidic acid ($C_{20:0}$)	0.4	0.3
Behenic acid ($C_{22:0}$)	0.1	0.1
Total	100	100

[1)] HWCS=heat-treated whole cottonseed at 85℃ for 30 min.

Table 4. *In vitro* digestibility of raw whole cottonseed (RWCS) and heat-treated whole cottonseed (HWCS)

	IVDMD[1)]	IVOMD[2)]
RWCS	48.81±1.69	52.61±1.01
HWCS[3)]	48.62±1.94	51.49±0.81

[1)] Dry matter digestibility by *in vitro* procedure (Tilly and Terry, 1963).
[2)] Organic matter digestibility value by *in vitro* procedure (Tilly and Terry, 1963).
[3)] HWCS = heat-treated whole cottonseed at 85℃ for 30 min.
Values are mean ± standard deviation (n = 6).

the average, followed by oleic acid ($C_{18:1}$) averaged 16%. Those three major fatty acids existing in WCS were also reported by Bertrand et al. (2005) and Berberich et al. (1996).

In a study on the nutritional effect of heating whole soybean Faldet et al. (1991) found that longer heating time resulted in more complete protection of feed protein by accelerating Maillard reaction. The effect of heat-treatment of whole linted cottonseed on OM digestibility has been inconsistent among authors. Heat-treatment of cottonseeds has either not affected OM digestibility (Pena et al., 1986) or decreased it (Pires et al., 1997). The decrease in nutrient solubility and degradability of roasted WCS may accelerate the passage of fiber of WCS origin to the abomasum (Mabjeesh et al., 2000). Heat treatment of WCS by roasting at relatively high temperature of 149℃ increased the amount of ruminally undegradable protein from 30.4 to 34.2% (Pires, 1997). Since main objective of heat treatment in this study was to eliminate germination ability, optimal heating temperature should be as lowest as possible considering its economic efficiency. Although heating level applied in the study was much lower than that used by other authors above mentioned, WCS heated at 85℃ for 30 min in the oven caused a significantly ($p<0.05$) lowered rumen degradability both in DM and CP as compared with raw WCS (Fig. 2 and Fig. 3). On the average, increase of protein degradability for more than 10% unit was obtained for heated WCS during the incubation within the rumen.

As seen in Table 5, relatively higher fraction B obtained from the *in situ* incubation to other ones reported (Pires et al., 1997; NRC, 2001) might be due to the 2 step fine grinding of WCS for sample preparation. Pires et al. (1997) observed a decrease of protein degradability by 3.8% unit from WCS roasted at 149℃ with steeping. In this study, heating WCS at 85℃ for 30min in the oven similarly lowered its RDP content by 3.3% unit.

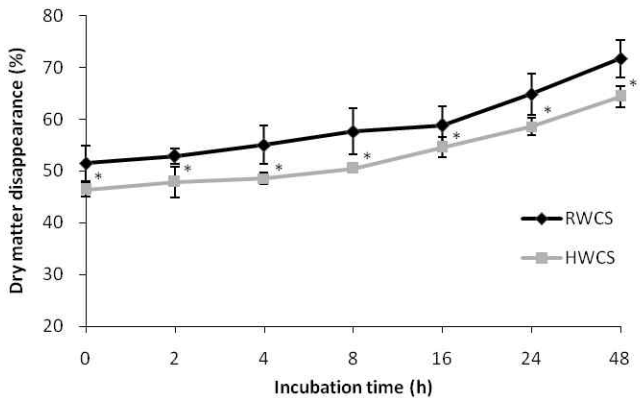

Fig. 2. *In situ* ruminal dry matter degradability for raw whole cottonseed (RWCS) and heat-treated whole cottonseed (HWCS).
* Mean values at each time were significantly different at $p<0.05$.

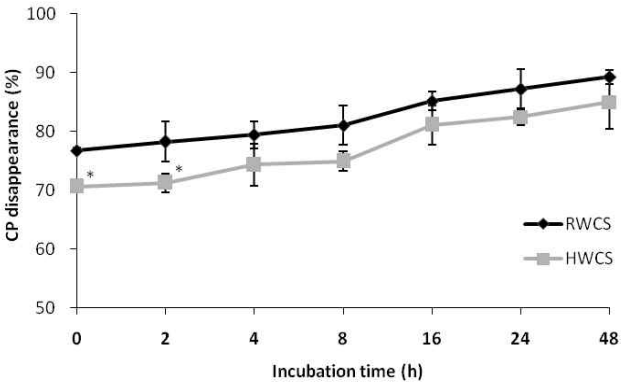

Fig. 3. *In situ* protein degradability for raw whole cottonseed (RWCS) and heat treated whole cottonseed (HWCS).
* Mean values at each time were significantly different at $p<0.05$.

Table 5. Fractions and degradation of protein *in situ* from raw whole cottonseed (RWCS) and heat-treated whole cottonseed (HWCS)

Item	RWCS	HWCS
Fraction A (washout), % of N	76.7	70.7
Fraction B, % of N	20.3	24.3
Fraction C, % of N remaining at 48 h	3.0	5.0
K_d, /h	0.055	0.060
K_p, /h	0.05	0.05
RUP, % of CP	12.7	16.0
RDP, % of CP	87.3	84.0

Fraction A (washout) = N leaving *in situ* bag after immersing the bags in warm water (39℃), fraction B = 100% minus fraction A minus fraction C, K_d = rate of digestion, and K_p = rate of passage (National Research Council, 1985).

Ⅳ. CONCLUSION

In conclusion, results obtained in the study indicate that heating condition used in this study, which was proved to be most appropriate and economic one to remove germination ability of WCS, may also improve nutritive

value for the ruminant with regard to the protection of protein in the cotton seed.

Ⅴ. ACKNOWLEDGEMENT

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