Effect of heat treatment duration on ruminal degradation and digestibility of whole nonlinted cottonseeds

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SUMMARY
The effect of the duration of the heat treatment of nonlinted whole cottonseeds (WCS) on the degradability of DM and CP in the rumen was determined using the in situ technique. Nonlinted whole cottonseeds were heated at 150°C for 60 (t1), 90 (t2) or 120 min (t3). N solubility was determined using autoclaved rumen fluid as a solvent. N solubility of WCS was: 36%, 24.14%, 16% and 9.37% for control, t1, t2 and t3 respectively, showing a quasilinear effect of the heat treatment. The degradation of DM and CP was determined in nylon bags suspended in the rumen for 2, 4, 8, 16, 24 and 48h. The heat treatments of whole cottonseeds decreased the ruminal degradability of DM (-17, -26, -36 units respectively) and CP (-5, -9, -14 units, respectively). Although the longest treatment (120 min at 150°C) gave the best protection for protein against rumen degradation, this could not be recommended as optimum as both nitrogen degradability and ADIN content expressed a linear evolution. Further studies, with longer heat treatments are needed in order to assess this optimum.

Keywords: cottonseeds, heat, degradation, protein

INTRODUCTION
Whole cottonseed (WCS) is a popular feedstuff for dairy cows, due to it is relatively high content in energy, digestible fiber and protein. Because of its high nutritional value, its incorporation into the diets of high producing dairy cattle is considerate beneficial. Its biological value is also higher than other vegetable proteins (Mabjeesh. 2000).

Large quantities of intestinally available protein are required by dairy cows in order to maintain high milk production. This can be enhanced by supplementing non degradable protein or by protecting dietary CP from excessive ruminal digestion. The ruminal degradation of crude protein (CP) from WCS is relatively high (H. Tagari, 1986). Reduction of this ruminal
degradation is preferable since it reduces N losses through excessive NH3 urea production.

Heat treatment of feedstuffs can decrease degradation of dry matter and crude protein by blocking reactive sites for microbial proteolytic enzymes (Broderick and Craig, 1980) and increase the supply of dietary protein to the duodenum (Tagari, 1986). Several studies (Faldet & Satter, 1991; Sahlu, 1984) on various protein sources have shown a correlation between decreased ruminal degradation of protein and increased milk production.

Various heat treatments are available for decreasing degradability of oilseeds: oven-heating, roasting, extruding and autoclaving. Heat treatment has the advantage of being safe, rather inexpensive and easily available (not requesting complex equipment). However, the knowledge on optimal conditions of heat treatments of cottonseeds is scarce. Whereas data on the effect of the intensity of the heat treatment there are present in the literature, we found no data on the effect of its duration.

The objective of this study was to determine the effect of the duration of heat treatment of WCS on DM and CP ruminal degradation and to find an optimum compromising between beneficial reduction of degradability and detrimental reduction of the digestibility of the ruminal by-pass.

**MATERIAL AND METHODS**

**Whole cottonseed treatment**

A variety of cottonseed from Syria (variety Aleppo 90 which was developed by the Cotton Bureau in Aleppo) were treated as follows: non-treated (C), heat treated at 150°C for 1h (T1), heat treated at 150°C for 1h30 min (T2), and heat treated at 150°C for 2h (T3). Heat treatment was applied in an oven in which WCS were spread on trays in 2 cm layers. After cooling, WCS was ground to pass through a 2-mm screen sieve.

**Animals and diets**

Three non lactating cows, fitted with ruminal cannulas, were fed a diet designed to meet maintenance requirements and to ensure optimum conditions for rumen microbial ecosystem. The diet fed in 2 meals/ day consisted of 5 kg of barley hay, 100g molasses and 2 kg of compound feed, composed of: 38.5% barley, 38.5% dry beet pulp, 18% sunflower meal, 2% calcium phosphate, 1.5% salt and 1.5% specific vitamin-mineral premix.

**N solubility and ADIN**

Soluble N was determinate by incubating approximately 1g of WCS in 100 ml of autoclaved rumen fluid at 400C for 2 h. Solubility was determined at pH 6.7. The rumen fluid used as a solvent represented sample collected from a fistulated dams on an ad libitum diet. Approximately 4 liters of rumen fluid were collected. Collections were strained through four layers of cheesecloth and
autoclaved at 120° C for 45 min. The autoclaved rumen fluid was then centrifuged (1,500 × g for 5 min) and refrigerated until use (J. F. Wohlt. 1972). After incubation samples were centrifuged at (1,500 × g for 5 min) and determined on 50 ml of the supernatant liquid by the macro-Kjeldahl method.

In situ incubation

Ruminal degradability was determined using in situ method, adapted from Michalet-Doreau et al, 1987 and Dulphy et al., 1999. Approximately 3 g of WCS were incubated in sewed nylon bags measuring 6 × 10 cm and having an average pores size of 50 µm. Bags were incubated for 0, 2, 4, 8, 16, 24 and 48 hours in the dorsal rumen. The bags for 2, 4 and 8 hours were inserted at the same time (T0), whereas the bags for 16, 24 and 48 hours were inserted at T8, after removal of the first bags. After removal, the bags were rinsed, stored in the freezer until completion of the series, mechanically treated for elimination of microbes attached to the undegraded particles, washed (in a washing machine) and dried at 65°C for 48 hours. The content of incubated bags were pooled per incubation time (3 repetitions, corresponding to the three fistulated cows), and analyzed for N content by Kjeldahl method.

Calculations

Data were corrected for microorganism firmly attached to dietary particles which are not removed through mechanical contamination, using the corrections proposed by Ould-Bah, 1989, then fitted with the nonlinear regression equation proposed by McDonald and Orskov, 1979: \( P = a + b(1 - e^{c * t}) \). The nonlinear parameters of this equation (a, b and c) were analyzed using a nlmixed procedure (SAS, 1996). Effective degradability, \( d(%) \) was calculated using a rate of solid outflow from the rumen of 0.06/ h (equivalent to dairy cow with a milk production of 15 l/d).

RESULTS AND DISCUSSION

The chemical compositions of WCS are: Dry Matter 97.56; Crude Protein 20.39; Fat 18.07; Cellulose 29.34; Ash 3.41. These values are usual for a typical commercial WCS. All heat treatment durations were followed by a reduction of the N solubility of WCS presented in Table 1. The extension of the duration of the heat treatment led to a considerably reduction of solubility, for each half hour added: \( Y(%) = 0.36 - 0.223 * X \), \( r^2 = 0.99 \), where x is expressed in minutes. As solubility only gives an overall image, the effect of treatments at ruminal and post-ruminal level were assessed through in situ and ADIN analyses, respectively.

Thus, heat treatment of WCS was associated with linear increase \( Y(%) = 10.23 - 0.053 * X \), \( r^2 = 0.99 \) of ADIN proportion in total nitrogen (Table 1). The variety of WCS used in this study has slightly high proportion of ADIN comparing with other varieties (Arieli, 1989). However, the increase of ADIN
after heat treatment is in agreement with other results (Arieli, 1989; Kung, 1983; Satter, 1986).

Table 1. Effect of the duration of heat treatment on nitrogen solubility and acid detergent insoluble nitrogen of whole cottonseeds nitrogen

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N solubility, % of total N</th>
<th>ADIN, % of total N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no heat treatment)</td>
<td>36</td>
<td>10.36</td>
</tr>
<tr>
<td>t1 (heated for 60 min at 150°C)</td>
<td>24.14</td>
<td>13.01</td>
</tr>
<tr>
<td>t2 (heated for 90 min at 150°C)</td>
<td>16</td>
<td>15.21</td>
</tr>
<tr>
<td>t3 (heated for 120 min at 150°C)</td>
<td>9.37</td>
<td>16.54</td>
</tr>
</tbody>
</table>

In situ disappearance of DM and CP is presented in figures 1 and 2. The heat treatment of WCS decreased the disappearance of DM and CP at all times of incubation. The parameters of rumen degradation and effective degradability were presented in tables 2 and 3. The parameters of rumen degradation and effective degradability were influenced by the heat treatment and the effective rumen degradations of WCS were reduced for all treatments. Thus, DM degradability decreased by -17, -26, and 36 units for t1, t2, and t3 respectively. CP degradability was also reduced but in a lower extent: -5, -9 and –14 units for t1, t2, t3 respectively.

Figure 1. Effect of the duration of heat treatment on dry matter disappearance of whole cottonseeds.

Heat treatment duration of WCS reduces the degradation of DM and CP, this effect being partly related to the blocking of sites reactive for microbial proteolysis enzymes and partly to the reduction of protein solubility (Broderick and Craig, 1980). This effect was clearer for the degradability of DM than for CP. For example, heating WCS at 150°C for 2 h has reduced the degradability
of DM with 36 units and only with 14 units for CP. This observation suggested that heat treatment of WCS would be associated not only with a reduction of protein degradation but also with a reduction in the ruminal degradation of carbohydrate. It has to be noted that an excessive unavailability of nutrients (e.g. following an excessive heat treatment) may be followed by a reduction of microbial synthesis in the rumen.

![Figure 2.Effect of the duration of heat treatment on crude protein disappearance of whole cottonseeds.](image)

Table 2. Effect of the duration of heat treatment on the degradability of dry matter of WCS*

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>t1</th>
<th>t2</th>
<th>t3</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>a**</td>
<td>34.91^a</td>
<td>26.43^b</td>
<td>37.38^a</td>
<td>25.66^cb</td>
<td>2.0583</td>
</tr>
<tr>
<td>b**</td>
<td>55.18^a</td>
<td>59.42^a</td>
<td>29.19^bc</td>
<td>37.16^c</td>
<td>2.5193</td>
</tr>
<tr>
<td>c**</td>
<td>18.39^a</td>
<td>10.19^b</td>
<td>11.46^ab</td>
<td>9.94^ch</td>
<td>2.1767</td>
</tr>
<tr>
<td>d</td>
<td>76.52</td>
<td>63.83</td>
<td>56.54</td>
<td>48.83</td>
<td></td>
</tr>
<tr>
<td>r^2</td>
<td>0.9654</td>
<td>0.9908</td>
<td>0.9921</td>
<td>0.9843</td>
<td></td>
</tr>
</tbody>
</table>

*a* means in rows with different superscripts differ (P< .05).

** a, b and c are nonlinear parameters, where a = rapidly degraded fraction, b = slowly degraded fraction degraded at rate c; r^2 is the coefficient of determination. Effective DM and CP degradabilities (d) are calculated on the basis of a ruminal outflow rate of .06/h.

An inconsistency was observed between N solubility and the degradability of CP and, more relevant, between N solubility and the “a” coefficients (table 2 and 3) representing the readily degradable part of WCS DM and CP, respectively. The intensive wash of the incubated bags, leading to lose of insoluble particle from all bags (containing treated or untreated WCS) probably
contributed to this inconsistency but other factors may be also involved. The evolution of N solubility confirmed the overall effects of heat treatments but could not be used in further analyses. On the other hand, ADIN is directly related to heat-induced damage and it is considered as representing unavailable CP (Thomas, 1982).

Table 3. Effect of the duration of heat treatment on the degradability of Crude Protein of WCS

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>t1</th>
<th>t2</th>
<th>t3</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>a**</td>
<td>73.52^a</td>
<td>67.84^bc</td>
<td>56.27^cd</td>
<td>44.32^d</td>
<td>9.2397</td>
</tr>
<tr>
<td>b**</td>
<td>24.43^a</td>
<td>29.99^bc</td>
<td>37.47^cd</td>
<td>47.72^d</td>
<td>9.9800</td>
</tr>
<tr>
<td>c**</td>
<td>23.61^a</td>
<td>12.33^b</td>
<td>18.56^cb</td>
<td>17.74^db</td>
<td>3.5839</td>
</tr>
<tr>
<td>r^2</td>
<td>0.9992</td>
<td>0.9693</td>
<td>0.9756</td>
<td>0.9965</td>
<td></td>
</tr>
</tbody>
</table>

*means in rows with different superscripts differ (P< .05).
** a, b and c are nonlinear parameters, where a= rapidly degraded fraction, b= slowly degraded fraction degraded at rate c; r^2 is the coefficient of determination. Effective DM and CP degradabilities (d) are calculated on the basis of a ruminal outflow rate of .06/h.

The duration of the heat treatment linearly reduced the nitrogen degradability (Y, % = 93.56 – 0.106 × X, r^2 = 0.97, where X is expressed in minutes), suggesting that prolongation of the heat treatment might additionally decrease the degradability. On the other hand, the duration of the heat treatment led to a linear ADIN evolution. These prevented identification of an optimal duration of heat treatment, compromising between protection of proteins against ruminal excessive degradation and the need for a highly digestible protein bypass.

CONCLUSIONS

Heat treatment of nonlinted WCS at 150°C for 60, 90 and 120 min linearly reduced in situ degradability of DM and CP. DM degradability was reduced by -17, -26 and -36 units for t1, t2 and t3 respectively and CP degradability was reduced by -5, -9 and -14 units for t1, t2 and t3 respectively. In this context, the best option is heating WCS for 120 minutes, unless economical reasons (e.g. driven by price of processing) interfere.

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