Influence of Commercial Insoluble-dietary Fibers on Digestibility and Protein Utilization by Rat Bioassays.

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ABSTRACT
Dietary fiber has been reported to decrease digestibility and utilization of many nutrients, including protein. The objective of this research work was to study the effect of commercial insoluble-dietary fibers have on the digestibility and protein utilization measured by rat bioassays. Three experimental diets were prepared containing 10\% casein and different source of commercial dietary fibers, at 5.0 \% level: oat fiber (OF), maize fiber (MF), soy fiber (SF); a control diet with cellulose fiber (CF) and a reference diet based on whole oat (WO), were also included. Neither of the experimental diets, except MF had effect on apparent (\% AND) and true (\% TND) nitrogen digestibility. Only the WO reference diet caused a decrease in AND and TND. These results demonstrate that commercial sources of DF, at 5\% level, did not reduce \% digestibility but caused a significant reduction in protein utilization, of casein-based diets, as measured by the NPR bioassay.

Keywords: Insoluble fiber, digestibility, NPR, Casein, Bioassay.
1. INTRODUCTION
Currently and after 30 years of research, dietary fiber (DF) is part of what is considered a healthy diet (Escudero & Gonzalez, 2006). Dietary fiber is classified into two main categories, insoluble (IDF) and soluble SDF. This grouping is based on chemical, physical, and functional properties. Soluble fiber dissolves in water forming viscous gels. They bypass the digestion of the small intestine and are easily fermented by the microflora of the large intestine. DF contains pectins, gums, inulin-type fructans and some hemicelluloses (Anderson et al., 2009). In the human GI tract, insoluble fibers do not form gels due to their water insolubility and fermentation is severely limited. Some examples of insoluble fibers are lignin, cellulose and some hemicelluloses (Kumar & Banerjee, 2010). Most DF containing foods include approximately one-third soluble and two-thirds insoluble fibers (Lattimer & Haub, 2010). Diets with a high content of fiber, such as those rich in cereals, fruits and vegetables have a positive effect on health (Anderson et al., 2009). This beneficial effect has been related to a decreased in the incidence of several types of diseases, due to its beneficial effects like: increasing feces volume; lowering the time of transit of food in the intestine; lowering cholesterol and glycemic levels; trapping substances that can be dangerous for the human organism (mutagenic and carcinogenic agents); and stimulating the proliferation of the intestinal flora (Dhingra et al., 2011). These benefits are attributed mainly to the fiber’s resistance to digestion, its ability to absorb and retain water, forming a network which occludes and fixes some organic substances, such as lipids and carbohydrates. However, the DF also traps other macromolecules such as proteins and minerals, made them unavailable to digestion and therefore decreasing their absorption (Wang et al., 2006; Zurita & Moya, 2007).
It has been shown that insoluble dietary fiber may decrease protein digestibility, which originates from an increase in fecal energy loss, which is currently unknown if accompanied by compensatory mechanisms of the other components of energy balance (Salas et al., 2008).
A number of investigations have been carried out to study the effect of dietary fiber on the digestibility of nutrients (Mongeau et al., 1989; Wang et al., 2006; Misurcova et al., 2010). Most authors report that DF contents can impair the apparent digestibility of nutrients. In particular, the effect of DF differs with the source and nature of the fiber and relates to its chemical composition, as well as to its physical and chemical properties (Pirman et al., 2009).
In this study the influence of commercial insoluble-dietary fibers, from different sources, on digestibility and utilization protein, was evaluated. The purpose of this research was to determine the effect of commercial insoluble-dietary fibers, extracted from grains, on the nitrogen digestibility and protein utilization, through rat bioassays.

2. MATERIALS & METHODS
Fibers. The characteristics of the insoluble fiber sources used to prepare the diets are shown in Table 1. The insoluble fibers were added to diets by substitution in equivalent amount of one of the dietary component (cornstarch).
Diets. Diets were prepared according AOAC (1990): protein 10.0 %, sugar 5.0 %, oil 8.0 %, fiber 5.0 %, vitamin mix 2.5 %, mineral mix 3.5% and cornstarch 69%.
One control diet was prepared with cellulose fiber (CF), and three experimental diets were made varying the source of insoluble fiber: oat (OF), maize (MF) and soy (SF). The protein source of these experimental diets was casein. A reference diet was prepared with whole oat (WO) as source of protein. Four protein-free diets were also prepared, according to basal diet from the AOAC (1990), varying the source of insoluble fiber: oat (OB), maize (MB), soy (SB) and cellulose (CB). Diets compositions are shown in Table 2.
Animals. 24 Sprague-Dawley rats were obtained at 21 days of age (Animal production unit of DIPA, Sonora University) with an average weighed between 45-60g. They were housed individually in wire cages at an
ambient temperature of 25°C and 70% relative humidity with a 12h light:dark cycle. Rats were fed with diets *ad libitum* for two days as adaptation period. After this rats were weighed and randomly arranged in 6 groups of four rats per group. The mean weights of the groups differed by less than 1.0 g. Rat groups were then assigned to experimental diets and the experimental period lasted 14 days. 

**Procedure.** Diets and water were fed *ad libitum*. Feces were collected from each rat starting from the 3th day. Fecal samples were kept frozen pending analysis. Food intake, food wastage, feces and animal weights were recorded periodically and also at the end of the experimental period. 

**Analytical methods.** Dietary fiber, in experimental diets was analyzed according to the recommended methodology of AOAC (1997), method (985.29). Feces were oven dried at 55°C for 24 hours. The nitrogen content from insoluble fibers, diets, feces and protein free diets was determined by a micro kjeldahl method (46-13) of AACC (2000). 

**Indicators.** The indicators of dry matter digestibility (%DMD), apparent (%AND) and true (%TND) nitrogen digestibility and net protein ratio (NPR) were calculated according a Bodwell (1977). 

### 2.1 Statistical analysis

Results of indicators of protein quality are reported as mean values of eight determinations ± SD. Results were analyzed by one-way ANOVA and Tukey mean comparison test at p<0.05 using an statistical program JMP, version 5.0 (SAS, 2002). 

#### 3. RESULTS

The effect of insoluble fiber on feed consumption, feces weight and nitrogen digestibility (apparent and true) is shown in Table 3. Rats fed with the control diet showed an slight increase in feed consumption and in the amount of feces, when compared those results in the experimental diets. Experimental diets did not show significant differences in these parameters. Rats fed with the reference diet (WO) showed an increase in feed consumption and a significant increase in the amount of feces, when compared to experimental diets containing the commercial insoluble fibers. This may be due to the balanced proportion of soluble and insoluble dietary fibers contained in the oat meal. Rats consuming the reference diet made of oatmeal also showed a significant increment in feed consumption. 

Experimental diets did not show a significant difference in % apparent nitrogen digestibility. However rats fed with soy insoluble fiber, though not significant, gave higher values of % AND. These rats showed the highest nitrogen consumption and lower feces nitrogen excretion. Only the group of rats fed with WO (reference) presented a significant decrease in % AND, due, most probably, to the significant increase in the amount of feces nitrogen. 

Results obtained from rats fed the protein-free diets, containing different sources of dietary fiber are shown in Table 4. Feed consumption was not significantly different among these groups of rats. Also the amount of feces was not different among groups of rats fed with OB, MB y SB diets. Rats fed with cellulose (CB) had a significant increase in the amount of fecal nitrogen, when compared to other sources of insoluble dietary fiber. Endogenous nitrogen, as part of the total fecal nitrogen, was higher in rats fed with the CB diet (Table 4). 

True nitrogen digestibility was not significantly different among diets containing different sources of insoluble dietary fiber. Only the WO diet showed a significant decrease in the % TND, most probably due to the higher amount of fecal nitrogen obtained for this diet. 

Figure 1 shows rats weight gain variation when fed with experimental diets (a) and nitrogen-free diets (b). Total weight gained for rats fed with casein diets varied among 39-46g. Apparently there is not a significant effect of commercial dietary fibers on total weight gained. The WO reference diet produced the lowest weight gained, due to the fact that in this diet the source of protein is oatmeal instead of casein. Weight gained results are in agreement with those found by Feddern *et al.* (2008) y Shah *et al.* (1986).
Nitrogen-free diets prepared with different sources of insoluble dietary fiber showed an average 0.7g/d weight loss, which are lower when compared to the cellulose based diet.

NPR values of experimental diets are shown in Figure 2. Diets containing oat and maize insoluble dietary fibers did not show significant differences in NPR. However, the diet containing soy insoluble dietary fiber gave the highest value of NPR. Experimental diets containing soy and cellulose sources of fiber had the highest values of NPR, but they were not significantly different between them. Shah et al. (1982) reported similar values for diets based on casein, when wheat bran was the source of fiber.

4. DISCUSSION

Total fecal nitrogen was not affected by the addition of different sources of insoluble dietary fiber. The increased amount in fecal nitrogen by rats fed with WO was probably due to the combination of IDF and SDF in oatmeal, producing a higher retention of water, an increase in the volume and weight of feces (Delorme et al., 1981; Delorme & Wojcik, 1982; García & Velasco, 2007).

In this study, rats consuming commercial insoluble fibers (OF, MF y SF) gave higher % AND than rats consuming the cellulose control diet. Den Hartog et al., (1988) studied the effect of cellulose on protein digestibility and they did not find a significant effect when cellulose is added, at the 5% level in casein-based diets. However, Sauer et al., (1991) reported a slight effect on % AND when cellulose is added at the 10%. Experimental diets used in this study were adjusted to the 5% total dietary fiber and perhaps this level of DF addition was not sufficient to exert an effect on % AND. The level of addition of DF may be an important factor in the effect of DF on nitrogen digestibility, causing an increase in fecal nitrogen and therefore lowering % AND (Tetens et al., 1996). This may explain those results obtained for the WO diet in which N consumption was higher and a significant increase of excreted N was observed. Renteria et al., (2008) working with oat-based diets and several levels of DF reported values of 86.2 % AND. Pedó et al., (1999) also found similar % AND values for oatmeal diets.

In our study % TND was not affected by commercial insoluble fibers. These results are in agreement with those found by Siri et al. (1994) and Shah et al. (1982) that reported values 91.8 y 90.8%, respectively, for casein-based diets and 10% cellulose as a source of fiber. Shah et al. (1982) feeding rats with N-free diets and various sources of fiber, found that cellulose (5%), lignin (6%) and wheat bran (5%) produced similar amounts of endogenous N by rats consuming casein-based diets. In this study the effect of DF was not observed in digestibility, though DF did cause a reduction in NPR values.

A special effect on % TND was found in this study for the WO diet. This diet caused a significant increase in the amount of fecal N. Since the endogenous N was not found significantly different among the various sources of DF, then this diet produced an increase in the excretion of dietary N and therefore a decrease in the % TND. This source of DF exerts a negative effect on protein digestion and a consequent increase in the fecal N derived from the dietary N.

In terms of the protein utilization, in our study, rats that consumed experimental diets with oat and maize (OF and MF), showed a significant decrease in the utilization of the protein, as measured by the NPR bioassay. This is when comparison is made of these experimental diets with the control diet having cellulose as a source of fiber. A possible explanation for this is that cellulose can be metabolized in the large intestine, producing short chain fatty acids, CO₂ and methane, which can be utilized as a source of energy, causing a slight efficiency in the utilization of the dietary N (Shah et al., 1982). An important consideration is that in our study all experimental diets were prepared using casein as a source of protein, therefore the amino acid balance which promoted the rat growth, and consequently traduced as an increase in body weight, was the same for all experimental diets. The only difference among experimental diets was the source of insoluble fiber. Those reduced values of NPR found for experimental diets containing OF and MF may be due to a direct effect of the fiber components and their physicochemical nature on the growing
pattern of the rat. Mongeau et al. (1989) reported on the effect of different sources of dietary fiber on the protein quality of various foods. They concluded that the various components of the DF, as well as their level of addition are important in establishing the impact of DF on protein quality. DF in cereals is made of hemicelluloses as mayor component; and dry legumes contain cellulose as a main component of DF. Another possible explanation for the NPR results obtained in our study is based on the N balance analysis. The experimental diet made with oat fiber (OF) showed a higher excretion of dietary N than the other experimental diets (MF and SF), with the soy fiber having the lowest excretion of dietary N. Soy fiber may have produced a little effect on protein digestion, allowing that larger amount of proteins being absorbed, with the consequent reduction in the fecal N, presenting higher values of NPR. On the contrary, oat fiber exerts a larger influence on protein digestion, traduced as a decrease in the protein absorption, with the consequent increase in the dietary fecal N and a correspondent reduction in NPR values. The experimental diet containing maize DF (MF) had a similar behavior as the oat DF, only that to a lower extent. These considerations may explain those NPR results obtained in our study for experimental diets made of oats and maize DF.

Another consideration to be made to explain those NPR results obtained in our study is through the analysis of results given by the N-free diets. N-free diets caused an overall reduction in the rat body weight. There is no actually a weight gained by rats consuming these N-free diets. In our study the cellulose N-free diet caused the largest lost in rats body weight. Since in the calculation of NPR values, the weight loss of the N-free diets is being considered, then a larger weight loss obtained by the correspondent N-free diet has to be traduces as an increment of NPR. This may explain why the casein-based diet containing cellulose as a source of fiber gave larger values of NPR, when compared to the rest of experimental diets.

In our study the WO diet showed a significant reduction in NPR values. The explanation for this is that in this reference diet the source of protein is oatmeal, which has a different amino acid composition than the other experimental diets, where the source of protein is casein.

5. CONCLUSIONS
Experimental casein-based diets in which different commercial insoluble DF (OF, MF and SF) were added, gave higher % of AND and TND. These insoluble fibers had no effect on the weight gained of rats, but produced a significant decrease in the NPR values, especially those insoluble DF made of oat and maize. Oat and maize insoluble fibers, when added to protein-free diets presented a lesser decrease in body weight, a probable reason for those NPR values. These commercial insoluble dietary fibers produced a significant decreasing effect on the protein quality of experimental casein-based diets. This effect is due to the increasing amount of fecal N produced. The higher fecal N is coming from the dietary N and not from endogenous N. Therefore, these sources of commercial insoluble DF had an effect on protein quality. This deleterious effect of insoluble fibers on protein quality continues to be proven at different levels of addition, since results of this study were obtained at 5.0% level.

6. ACKNOWLEDGMENTS
The authors show their appreciation to the personal of the Animal Experimental Unit of the Department of Research and Graduate Studies for their collaboration on the rat bioassays. The financial support from the Head of the department of the Department of Research and Graduate Studies, as well as the financial support from the Chemical and Biology Division are also acknowledged.
7. REFERENCES


### TABLES WITH CAPTIONS

**Table 1.** Chemical composition of commercial insoluble-dietary fibers

<table>
<thead>
<tr>
<th>Insoluble fiber</th>
<th>Insoluble fiber(^1)</th>
<th>Soluble fiber(^1)</th>
<th>Protein(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oat</td>
<td>89.1±0.01</td>
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<tr>
<td>Maize</td>
<td>92.8±0.05</td>
<td>1.1±0.10</td>
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<td>Soy</td>
<td>87.2±0.30</td>
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<td>2.2</td>
</tr>
<tr>
<td>Cellulose</td>
<td>98.0±0.01</td>
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<td></td>
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<tr>
<td>Whole Oat</td>
<td>12.1±0.22</td>
<td>8.9±0.09</td>
<td>14.2</td>
</tr>
</tbody>
</table>

Dry weight basis. All values are expressed as mean ±SD of three determinations.
\(^1\)Dietary fibers were analyzed according to AOAC methodology (method 985.29).
\(^2\) micro-Kjeldahl AACC, 2000 (method 46-13).
Table 2. Composition of experimental and protein free diets.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>OF</th>
<th>MF</th>
<th>SF</th>
<th>CF</th>
<th>WO</th>
<th>OB</th>
<th>MB</th>
<th>SB</th>
<th>CB</th>
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<td>Casein</td>
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<td>112.5</td>
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<tr>
<td>Whole oat</td>
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<tr>
<td>Cornstarch</td>
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<td>577.5</td>
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<td>690</td>
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<td>Sugar</td>
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<td>Soy</td>
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<tr>
<td>Cellulose</td>
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<tr>
<td>Whole oat</td>
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<tr>
<td>Minerals</td>
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</table>

Table 3. Effect of insoluble-dietary fibers on biological indicators of experimental casein-based diets.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Food Intake (g)</th>
<th>Feces Weight (g)</th>
<th>Nitrogen intake (g)</th>
<th>Fecal nitrogen (%)</th>
<th>DMD&lt;sup&gt;1&lt;/sup&gt; (%)</th>
<th>AND&lt;sup&gt;2&lt;/sup&gt; (%)</th>
<th>TND&lt;sup&gt;3&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OF</td>
<td>140.6±13.9a</td>
<td>8.6±0.7a</td>
<td>2.32±0.2a</td>
<td>0.27±0.03a</td>
<td>93.8±0.4ab</td>
<td>88.5±0.6a</td>
<td>90.8±0.7a</td>
</tr>
<tr>
<td>MF</td>
<td>144.8±13.0a</td>
<td>8.4±0.9a</td>
<td>2.30±0.2a</td>
<td>0.26±0.04a</td>
<td>94.2±0.2a</td>
<td>88.9±1.0a</td>
<td>91.2±1.0a</td>
</tr>
<tr>
<td>SF</td>
<td>148.7±20.7a</td>
<td>9.1±1.1a</td>
<td>2.34±0.3a</td>
<td>0.25±0.03a</td>
<td>93.8±0.8ab</td>
<td>89.3±0.8a</td>
<td>91.4±0.7a</td>
</tr>
<tr>
<td>CF</td>
<td>149.5±21.2a</td>
<td>9.9±1.3a</td>
<td>2.31±0.3a</td>
<td>0.27±0.03a</td>
<td>93.1±0.8b</td>
<td>88.2±1.2ab</td>
<td>90.6±0.9a</td>
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<tr>
<td>WO</td>
<td>153.4±12.1a</td>
<td>16.0±1.4b</td>
<td>2.65±0.2a</td>
<td>0.35±0.04b</td>
<td>89.6±0.4c</td>
<td>86.9±0.7b</td>
<td>89.1±0.8b</td>
</tr>
</tbody>
</table>

All values are expressed as mean ±SD of eight determinations. Values with different subscript letter in the same column are significantly different (p<0.05). 1MD, dry matter of digestibility. 2AND, apparent nitrogen digestibility. 3TND, true nitrogen digestibility.

Table 4. Effect of commercial insoluble dietary fibers on protein-free diets.

<table>
<thead>
<tr>
<th>Basal diets</th>
<th>Food intake (g)</th>
<th>Feces Weight (g)</th>
<th>Feces nitrogen (%)</th>
<th>Endogenous nitrogen (g)</th>
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</thead>
<tbody>
<tr>
<td>OB</td>
<td>56.7±9.3a</td>
<td>2.7±0.6b</td>
<td>1.65±0.9a</td>
<td>0.051±0.006a</td>
</tr>
<tr>
<td>MB</td>
<td>56.9±8.6a</td>
<td>2.9±0.3b</td>
<td>1.59±0.4a</td>
<td>0.053±0.006a</td>
</tr>
<tr>
<td>SB</td>
<td>54.4±4.9a</td>
<td>3.1±0.2b</td>
<td>1.58±0.3a</td>
<td>0.049±0.002a</td>
</tr>
<tr>
<td>CB</td>
<td>56.8±7.6a</td>
<td>3.9±0.7a</td>
<td>1.55±0.1a</td>
<td>0.054±0.008a</td>
</tr>
</tbody>
</table>

All values are expressed as mean ±SD of eight determinations. Values with different subscript letter in the same column are significantly different (p<0.05)
FIGURE CAPTIONS

Figure 1. Effect of commercial insoluble dietary fibers on the body weight of rats fed with experimental casein-based diets (a) and N-free diets (b). Oat fiber (OF), maize fiber (MF), soy fiber (SF), cellulose fiber (CF), whole oat (WO), oat basal (OB), maize basal (MB), soy basal (SB) and cellulose basal (CB). All values are expressed are mean ±SD of eight determinations.

Figure 2. Effect of commercial insoluble dietary fibers on Net Protein Ratio (NPR). Values are expressed are mean of eight determinations. Values with different letter are significantly different (p<0.05).