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Effect of agave fructan, resistant maltodextrin and cellulose dietary fibers in gluten-based diets on protein digestibility and nitrogen utilization *in vivo*

Efecto de las fibras dietéticas fructano de agave, maltodextrina resistente y celulosa en dietas a base de gluten sobre la digestibilidad y la utilización de nitrógeno *in vivo*

Efeito das fibras alimentares frutano de agave, maltodextrina resistente e celulose em dietas à base de glúten na digestibilidade e utilização de nitrogênio *in vivo*

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Abstract

Dietary fiber intake improves the digestion process and decreases glucose and cholesterol levels. However, it could decrease protein digestibility. Cellulose is an insoluble fiber of the cell wall of plants. Resistant maltodextrin is a soluble fiber preceding resistant starch. Agavin is a soluble fiber obtained from agave mezontle and stems. Considering the consumption of low-quality protein diets increases year by year, this research was aimed at assessing the effect of ingesting 5 % cellulose, Agavin and resistant maltodextrin on protein digestibility and nitrogen utilization for growth on 10 % gluten-based and casein-based diets through *in vivo* assay using Sprague Dawley rats. Food consumption, weight gain, and fecal excretion were measured every third day for 14 days. The nutritional quality and the better acceptance of casein triggered higher rat weight increase. The lack of protein caused lower food consumption and rat weight loss. Gluten-based diets prompted intermediate food intake and low rat weight increase. Zero or five percent of dietary fiber did not cause a relevant effect on rat weight. So, the consumption of 5 % dietary fiber can help improve nutritional habits without affecting nitrogen intake for growth. Finally, Agavin as a dietary fiber represents a suitable option for the agave industry.

Keywords: functional food; Sprague Dawley; net protein ratio; NPR

Resumen

La ingesta de fibra dietética mejora el proceso de digestión y disminuye niveles de glucosa y colesterol. Sin embargo, podría disminuir la digestibilidad proteica. La celulosa es una fibra insoluble de la pared celular de las plantas. La maltodextrina resistente es una fibra soluble precedente al almidón resistente. Agavin es una fibra soluble obtenida de tallos y mezontle del agave. Considerando que el consumo de dietas con proteínas de baja calidad aumenta año con año, está investigación tiene como objetivo evaluar el consumo de 5 % de celulosa, Agavin y maltodextrina resistente en la digestibilidad proteica y utilización de nitrógeno para el crecimiento en dietas con 10 % de gluten y caseína mediante ensayo *in vivo* utilizando ratas Sprague Dawley. El consumo de alimento, peso ganado, y excreción fecal fueron medidos cada tercer día durante 14 días. El valor nutricional y la mejor aceptación de la caseína desencadenaron un mayor aumento en peso de las ratas. La ausencia de proteína causo el menor consumo de alimento y pérdida en peso de las ratas. Las dietas a base de gluten provocaron una ingesta intermedia de alimento y un bajo incremento en peso de las ratas. Cero o 5 % de fibra dietética no causó un efecto relevante en el peso de las ratas. Así, el consumo de 5 % de fibra dietética puede ayudar a mejorar los hábitos nutricionales sin afectar el nitrógeno ingerido para el crecimiento. Finalmente, el Agavin como fibra dietética representa una opción adecuada para la industria del agave.

Palabras clave: alimento funcional; Sprague Dawley; razón neta de proteína; NPR

Resumo

A ingestão de fibras alimentares melhora o processo de digestão e diminui os níveis de glicose e colesterol. No entanto, pode diminuir a digestibilidade das proteínas. A celulose é uma fibra insolúvel da parede celular das plantas. A maltodextrina resistente é uma fibra solúvel que precede o amido resistente. Agavin é uma fibra solúvel obtida a partir de caules e mezontle de agave. Considerando que o consumo de dietas com proteínas de baixa qualidade aumenta ano a ano, esta pesquisa teve como objetivo avaliar o consumo de 5 % de celulose, Agavin e maltodextrina resistente na digestibilidade das proteínas e a utilização de nitrogênio para crescimento em dietas com 10 % de glúten e caseína por ensaio *in vivo* usando ratos Sprague Dawley. O consumo de alimentos, o peso ganho e a excreção fecal foram medidos a cada três dias durante 14 dias. O valor nutricional e a melhor aceitação da caseína desencadearam um maior aumento de peso dos ratos. A ausência de proteína causou menor consumo de alimentos e perda de peso dos ratos. As dietas à base de glúten causaram ingestão alimentar intermediária e baixo ganho de peso dos ratos. Zero ou 5 % de fibra alimentar não causou efeito relevante no peso dos ratos. Assim, o consumo de 5 % de fibra alimentar pode ajudar a melhorar os hábitos nutricionais sem afetar a ingestão de nitrogênio para o crescimento. Finalmente, o Agavin como fibra dietética representa uma opção adequada para a indústria do agave.

Palavras-chave: alimento funcional; Sprague Dawley; razão proteica líquida; RPL

Introduction

Dietary fiber (DF) is a carbohydrate polymer of ten or more monomeric units (Anderson et al., 2009), and it is classified into two main categories, soluble (SDF) and insoluble (IDF) dietary fibers (Lattimer and Haub, 2010). Both hold some organic molecules and avoid their absorption (Adams et al., 2018). Thus, DF is a natural food component that can help lower the incidence and prevalence of obesity, type 2 diabetes, different cancers, and cardiovascular diseases (Lattimer and Haub, 2010). However, most people consume a lower daily amount of DF (10-18.8 g/day) than the 25-35 g/day intakes recommended by the World Health Organization (WHO) (Alfawaz et al., 2019; Thompson et al., 2020).

Researchers in food science and technology have studied natural ingredients for developing new functional foods with high DF content in response to consumer demand for more fiber-rich foods using ingredients such as cellulose, resistant maltodextrin and fructans, among others (Raninen et al., 2011; Allsopp et al., 2013; Santiago-García et al., 2021). Cellulose (CEL) is an IDF that makes up the highest proportion of biomass in the cell walls of most plants. It is considered the world’s most abundant homopolymer (beta-glucan polysaccharide). Resistant maltodextrin (RMD) is a SDF preceding starch. Both are widely used as fiber sources for human consumption and in animal trials (Lattimer and Haub, 2010; Abellán Ruiz et al., 2016). Fructan obtained from agave plants is called Agavin (AGV) (Lopez et al., 2003). It is a SDF obtained from agave mezontle and stems (Montañez-Soto et al., 2011) with good potential as a prebiotic and functional food for health, wellbeing and products enhancement (Santiago-García et al., 2021; Zimmermann et al., 2021). *Agave tequilana* Weber var. Azul is an economically important source of prebiotics used by the tequila industry (Lopez et al., 2003). Agave mezontle is used for tequila production, and stem residues can be exploited as fructan resources. This could allow using the whole Agave plant (Montañez-Soto et al., 2011).

The resistance of DF to digestion and its water absorption and retention capacities are associated with health benefits. The consumption of DF promotes disorders in the absorption of nutrients, and it is associated with lower levels of total lipids, total cholesterol, and low-density lipoproteins. However, it also could decrease protein digestibility and nitrogen utilization (Zhang et al., 2013; Adams et al., 2018; García-Curbelo et al., 2018; Opazo-Navarrete et al., 2019). Formulations with Agavin have antioxidant and protective roles in the brain. They produce beneficial changes in lipid metabolism, reduce body weight caused by high-fat diets, and can produce an anorexigenic effect (Franco-Robles and López, 2016; García-Curbelo et al., 2018). On the other hand, most people eat a high quantity of food with low-quality plant-based protein such as wheat gluten, which is part of many foods of daily consumption such as bread, pasta, cookies, and tortillas (Henchion et al., 2017). Besides, utilization of gluten has increased up to 1.8 % annually in the last 30 years (Lerner et al, 2017).

Considering that low-quality protein diets are increasing year by year, the effects of DF on protein digestibility could affect the basal N for humans and animals' growth, increasing N excretion. This research aimed to assess the effects of ingesting 10 % wheat gluten-based diets containing 5 % IDF or 5 % SDF on protein digestibility and nitrogen utilization for Sprague Dawley rats' growth. The results are expected to provide information on whether the recommended percentage of DF and wheat gluten as a protein source can enhance or decrease protein utilization for body maintenance and growth. That information, in turn, would be useful for looking at the proper amount of DF in food formulations to help promote wellness and healthy body weight. In addition, the use of Agavin as SDF encourages the use of all parts of Agave plants.

Experimental design

The experiment was designed according to the recommendations of Alfaro (2005), Rucker and Watkins (2019), and the official NOM-062-ZOO-1999 (de Aluja, 2002). All these focus on animal welfare and generate reliable and replicable data. The 3 R's of the humanitarian technique –replace, reduce, and refine– were applied in this research to reduce the number of animals used in the study and to obtain reliable data.

The Animal Experimental Laboratory of the Department of Research and Graduate Studies in Food at the University of Sonora furnished the rats and the infrastructure to develop the bioassays. The formulation of experimental diets and the design of experimental conditions were carried out according to Bender and Doell (1957) and FAO/WHO guidelines (1991).

Raw Materials

Commercial Blue Agave fructan (Agave tequilana Weber var. Azul) (YASIN, Mexico), resistant maltodextrin (FIBERSOL-2, Decatur, IL, U.S.A.), and cellulose (Cellulose FCC, Bethlehem, PA, U.S.A.) were used as DF. Commercial wheat gluten (Seitan, Mexico) and casein (Product No. C7078; Sigma Chemical Co., St. Louis, MO) were employed as protein sources. In addition, commercial oil (Maceite, GDL, Mexico), sucrose (Zulka, SIN, Mexico), vitamin mix (AIN-76A, Dyets inc., Bethlehem, PA, U.S.A.), mineral mix (AIN-76, Dyets inc., Bethlehem, PA, U.S.A.), and 100 % natural cornstarch (CAZEL, Mexico) were used to complete formulations.

Chemical Analysis of Raw Materials

Experimental diets were formulated based on the chemical composition of the raw material. The moisture (method 925.10) and protein (factor 6.25, method 960.52) content of wheat gluten were determined following the procedures of the AOAC (2000). The fiber content from the commercial fiber sources was determined with a SIGMA-ALDRICH (DF-100A) enzymatic kit, using gravimetric method 985.29 (AOAC, 2000).

Digestibility and Net Protein Ratio Assay

Formulation of Experimental Diets

The experimental diets were formulated to contain 5% DF and 10% protein. AGV, RMD, and CEL were used as sources of DF. Commercial wheat gluten (GLU) and casein (CAS) were employed as protein sources.

Three gluten-based diets with Agavin (GLU-AGV), resistant maltodextrin (GLU-RMD), and cellulose (GLU-CEL) were formulated. In addition, three control diets were used: free-fiber (GLU-FFD), casein-based (CAS-CEL), and nitrogen-free (NFD-CEL) diets.

AGV is an economic DF, and it is a development option for the agave industry. CEL and RMD are widely used as DF sources, not only for human consumption but also for animal trials. Finally, these three DF participate differently in the digestive processes due to their structure and solubility in water. GLU-FFD was used to relate lack of DF to the digestibility and utilization of low-quality protein sources such as gluten.

GLU is a low-quality protein widely used in human diets. CAS is a high-quality protein source and serves as an indicator of the maximum protein efficiency value for digestibility and growth. NFD was prepared to obtain the cost of the metabolic nitrogen and to calculate the amount of nitrogen used only for weight gain. The CAS-based diet and the NFD were formulated using CEL because this DF does not interfere with nitrogen utilization (Falcón-Villa et al., 2014).

Table 1 shows the ingredients used to formulate each diet. All the nutrients were blended in a Hobart mixer (Hobart Corp, model A5 2001) until homogenization and stored in polyethylene bags at 4°C.

Table 1. Formulation of the experimental and control diets.1

Diet	Casein	Gluten	Fiber	Sucrose	Oil	Vitamin[2]	Mineral[3]	Wheat Starch
					($g\ Kg^{-1}$)			
GLU-AGV	-	128,2	50	120	80	25	35	561,8
GLU-RMD	-	128,2	50	120	80	25	35	561,8
GLU-CEL	-	128,2	50	120	80	25	35	561,8
GLU-FFD	-	128,2	-	120	80	25	35	611,8
CAS-CEL	112,5	-	50	120	80	25	35	577,5
NFD-CEL	-	-	50	120	80	25	35	690

[1]Diets were designed to satisfy the nutritional requirements of experimental animals (NRC 1995). All diets contained 3.960 kcal Kg^{-1}.
[2]Vitamin mix composition: Thiamin, riboflavin, pyridoxine, niacin, calcium pantothenate, folic acid, biotin, cyanocobalamin, menadione sodium bisulfite, vitamin A palmitate, vitamin E acetate, vitamin D3, vitamin D2, ascorbic acid, inositol, choline, p-aminobenzoic acid, niacinamide, and vitamin K1.
[3]Mineral mix composition: Ca, P, K, Na, Cl, S, Mg, Fe, Cu, Mn, Zn, Cr, I, and Se.

Design of Animal Experimental Groups and Bioassay Conditions

Groups of experimental rats were set up according to literature (Bender and Doell, 1957) and randomly assigned to each experimental and control diet. All the experimental animals were kept free from zoonotic diseases and external parasites. The animals were handled carefully to avoid altering their metabolic state or inducing a state of shock.

The test groups consisted of 24 Sprague Dawley rats between 21-23 days of age and 45-55 g in weight. Each animal test group consisted of two males and two female rats, with an average weight not exceeding 1 g between each of them. The animals were housed in individual stainless-steel cages with water and food ad libitum. A paper tray was placed under the cage for the collection of feces.

The experimental animals were maintained under controlled conditions at 25°C, 60-80% relative humidity, 12:12-h light-dark cycles, acclimated to the environment for two days and submitted to evaluation for 14 days. Food consumption, weight gain, and fecal excretion data were recorded every three days.

Protein Digestibility and Nitrogen Utilization for Growth Determination

DF intake, protein intake, and N intake were calculated based on food consumption and the quantity of protein in the corresponding diet. After drying and milling the feces, the protein content was determined by micro Kjeldahl (factor 6.25, method 960.52) (AOAC, 2000) to obtain the N excretion value. The values of dry matter digestibility (DMD), apparent protein digestibility (APD), true protein digestibility (TPD), and net protein ratio (NPR) were calculated using the following equations (Bender and Doell, 1957):

$$\% \, DMD = \frac{Food\ consumption\ (g) - Feces\ excretion\ (g)}{Food\ consumption\ (g)}\ x\ 100$$

$$\% \, APD = \frac{N\ intake\ (g) - N\ excretion\ (g)}{N\ intake\ (g)}\ x\ 100$$

$$\% \, TPD = \frac{N\ intake\ (g) - (N\ excretion\ (g) - N\ excretion\ by\ NFD\ (g))}{N\ intake\ (g)}\ x\ 100$$

$$NPR = \frac{Weight\ gain\ (g) - Weight\ gain\ by\ NFD\ (g)}{Protein\ (N\ x\ 6.25) consumption\ (g)}$$

Since each specific bioassay could underestimate or overestimate the NPR measure. NPR values were corrected to the standard value of 4.34 (cNPR). The cNPR value was calculated as follows:

$$cNPR = \frac{4.34}{NPR\ average\ of\ the\ casein\ control\ diet} x\ NPR\ of\ each\ diet$$

Statistical Analysis

Results were expressed as mean values ± standard deviation. Data were subjected to one-way analysis of variance (ANOVA) following general model procedures.

The protein quality parameters of each diet were compared with Tukey's test using the SAS program (2018, version 14, SAS Institute, Cary, NC, USA). In all cases, the mean values were considered significantly different at $P \leq .05$.

Results

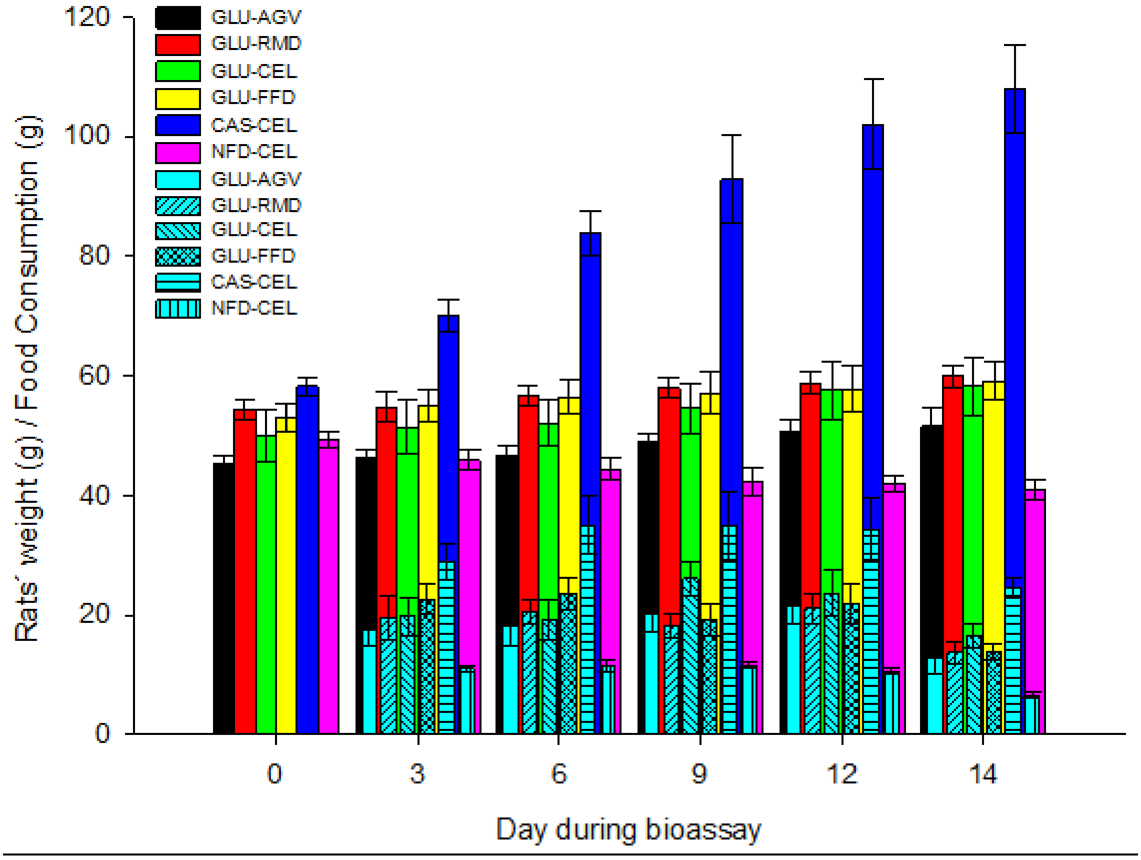
Chemical Analysis of Raw Materials

Commercial wheat gluten and casein had 78% and 88.8% protein content, respectively. True fiber of AGV, RMD, and CEL was 85.7%, 90.0%, and 98% , respectively. The results were used to formulate the experimental and control diets containing 10 % protein and 5 % DF (Table 1). All diets provided 3,960 kcal/ kg and 12.63 g of DF/1000 kcal, except for GLU-FFD.

Food consumption and Fecal Excretion related to DF Intake and Weight Gain through Time

Figures 1 and 2 show the food consumption, weight gain, and fecal excretion of Sprague Dawley rats during the in vivo assay, respectively. Table 2 displays the total amount of each parameter measured at the end of the bioassay.

Figure 1 shows the amount of food ingested and the changes in weight for groups of rats fed with each of the diets analyzed. Bars of the same color show weight changes in rats that consumed the same diet during the bioassay. The light blue bars represent the amount of food consumed by rats during the assay, thus relating food consumption to rat weight for each diet.



Note: Full-colored bars represent the weight of rats during the bioassay. The weight at day zero is according to the initial weight of the groups of rats. Light blue bars with lines represent food consumption by rats during the assay. A light blue bar in front of a full-colored bar relates food consumption to rat weight for each diet. There are no light blue bars at day zero because diet intake began at this time.

Figure 1. Values recorded per day of food consumption and weight gain by the Sprague Dawley rats during the *in vivo* assay.

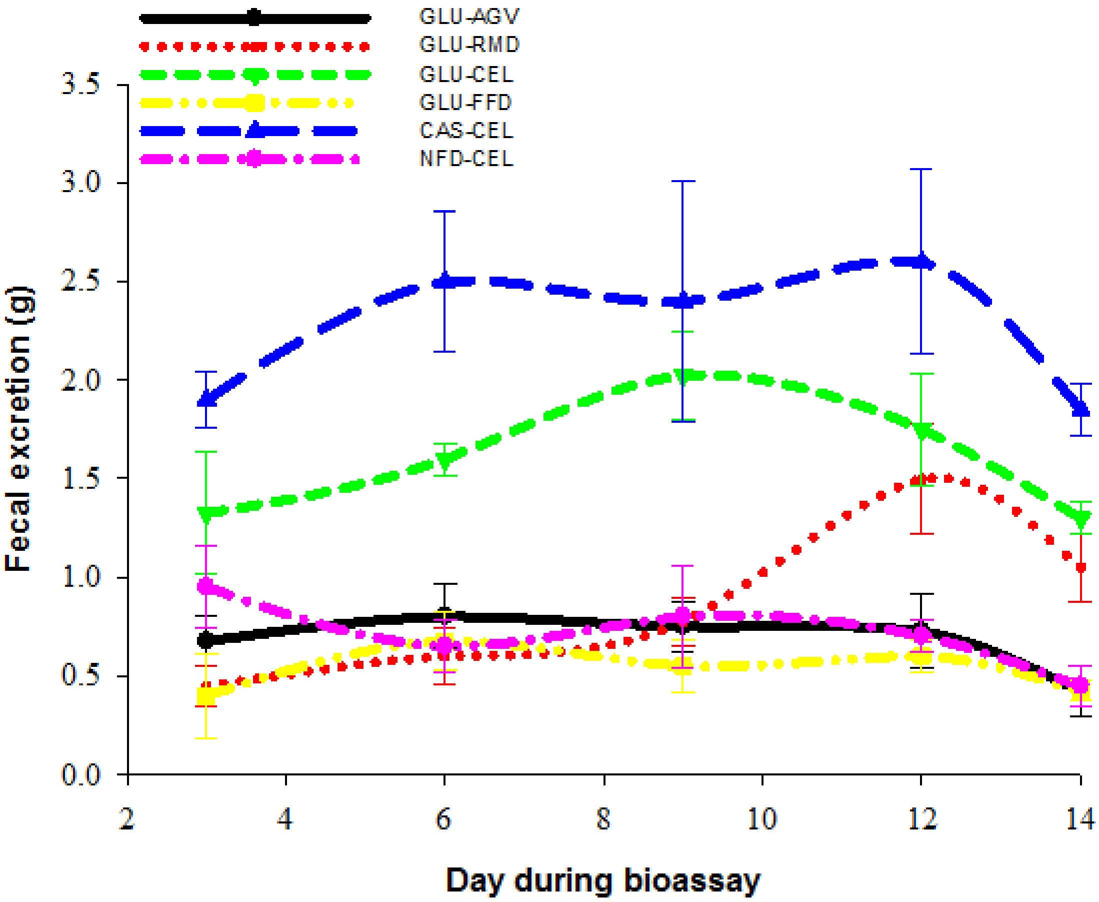


Figure 2. Daily fecal excretion by Sprague Dawley rats during the *in vivo* assay.

Table 2. Parameters obtained from the *in vivo* assay using Sprague Dawley rats.[1]

Diets	Food consumption (g)	Dietary fiber intake (g)	N intake (g)	Weight gain (g)	Fecal excretion (g)	Total N excretion (g)[3]	Dietary N excretion (%)
GLU-AGV	89,92 ± 13,26^{b}	3,85 ± 0,56^{c}	1,52 ± 0,22^{b}	6,28 ± 2,38^{b}	3,38 ± 0,45^{cd}	0,16 ± 0,02^{bc}	6,44 ± 0,72^{a}
GLU-RMD	93,17 ± 10,36^{b}	4,19 ± 0,46^{bc}	1,65 ± 0,18^{b}	5,55 ± 1,23^{b}	4,38 ± 0,28^{c}	0,18 ± 0,01^{b}	7,15 ± 1,14^{a}
GLU-CEL	105,25 ± 13,88^{b}	5,16 ± 0,68^{b}	1,72 ± 0,22^{b}	8,33 ± 1,20^{b}	8,00 ± 0,74^{b}	0,12 ± 0,01^{cd}	3,43 ± 0,23^{b}
GLU-FFD	101,13 ± 10,56^{b}	ND[2]	1,71 ± 0,17^{b}	6,15 ± 2,53^{b}	2,65 ± 0,34^{d}	0,11 ± 0,01^{d}	3,09 ± 1,15^{b}
CAS-CEL	157,95 ± 11,21^{a}	7,74 ± 0,54^{a}	2,43 ± 0,17^{a}	49,70 ± 6,49^{a}	11,30 ± 1,46^{a}	0,30 ± 0,03^{a}	7,94 ± 1,02^{a}
NFD-CEL	51,40 ± 1,19^{c}	2,52 ± 0,05^{d}	ND[2]	´-8,40 ± 1,37^{c}	3,55 ± 0,44^{cd}	0,06 ± 0,01^{e}[4]	ND[2]

[1]Values are expressed as mean ± standard deviation. Mean values followed by the same lowercase in the same column are not significantly different (*P* ≤ .05).

[2]ND= Not detected.

[3]Metabolic and dietary nitrogen excretion by Sprague Dawley rats.

[4]Metabolic nitrogen excretion by Sprague Dawley rats.

CAS-CEL and NFD-CEL were the most and the least consumed diets by rats, respectively. Besides, fecal excretion seemed to be related to food consumption and affected by the type of

DF. The incorporation of casein improved diet ingestion. As a result, rats feeding with CAS-CEL displayed the highest quantity of fecal excretion during the assay. The incorporation of DF triggered a heightened fecal excretion by the rats, with IDF in particular supporting a higher increase of fecal excretion than SDF. While deprivation of dietary nitrogen resulted in little food consumption by rats, reflected in low fecal excretion during the assay. In the end, the type of DF affected the percentage of fecal excretion. The three groups of rats fed with CEL (IDF) displayed a higher proportion of fecal excretion (7-7.6 %), followed by rats fed with SDF (3.8-4.7 %) and finally by rats fed with FFD (2.6 %).

All groups of rats triggered a high percentage of N absorbed (89.8-97.7 %) related to nitrogen intake and nitrogen excreted. Rats fed with CAS-CEL and SDF excreted a higher percentage of dietary nitrogen followed by rats fed with GLU-CEL and GLU-FFD. So, rats fed with GLU-CEL and GLU-FFD absorbed a slightly higher percentage (97.7 %) of dietary nitrogen than CAS-CEL and SDF (96 % and 90-96 %, respectively). Rats fed with NFD-CEL excreted a small quantity of endogenous, not dietary, nitrogen.

As mentioned, the incorporation of CAS improved food acceptability. A large proportion of the dietary nitrogen was absorbed, despite the high level of dietary nitrogen excreted or the high amount of DF ingested. Consequently, rats feeding on CAS-CEL increased their weight rapidly and at a higher proportion than the others. These animals had an average weight increase of 83% compared to GLU-CEL, and 88% compared to GLU-FFD and GLU-based diet with SDF (GLU-AGV and GLU-RMD). In contrast, the lack of dietary nitrogen for growth and the lowest acceptance of NFD-CEL caused the lowest ($P \leq .05$) DF consumption and weight loss (-8.40 g). Instead, the rats feeding with GLU-based diets ingested similar ($P \leq .05$) food and gained a comparable weight among them. These experimental animals gained weight slowly and achieved a low weight gain.

Dry Matter Digestibility of Diets

Table 3 displays the DMD values of the experimental diets. Although DMD was more affected by the addition of IDF than by the incorporation of SDF, all diets showed good DMD, higher than 92 %. In addition, the lack of DF on diets prompted the highest DMD value.

Neither the type of protein nor the absence of protein had a relevant effect on DMD. GLU-CEL, CAS-CEL and NFD-CEL diets had the lower DMD.

Table 3. Results of dry matter digestibility (DMD), apparent and true protein digestibility (APD and TPD), and corrected Net Protein Ratio (cNPR) of gluten- and casein-based diets.[1]

Diets	DMD (%)	APD (%)	TPD (%)	cNPR
GLU-AGV	96,23 ± 0,28[ab]	89,74 ± 0,78[b]	93,51 ± 0,72[b]	1,75 ± 0,17[b]
GLU-RMD	95,28 ± 0,37[b]	90,02 ± 1,39[b]	93,46 ± 1,14[b]	1,54 ± 0,20[b]
GLU-CEL	92,37 ± 0,35[c]	93,16 ± 0,31[a]	96,50 ± 0,22[a]	1,78 ± 0,15[b]
GLU-FFD	97,38 ± 0,21[a]	93,54 ± 1,19[a]	96,86 ± 1,14[a]	1,53 ± 0,14[b]
CAS-CEL	92,90 ± 0,49[c]	89,73 ± 0,88[b]	92,06 ± 1,02[b]	4,34 ± 0,23[a]
NFD-CEL	93,08 ± 1,01[c]	ND[2]	ND[2]	ND[2]

[1]Values are expressed as mean ± standard deviation. Mean values followed by the same lowercase in the same column are not significantly different (*P* ≤ .05).

[2]ND= Not detected.

Protein Digestibility and Nitrogen Utilization for Growth Determination

Protein Digestibility of Diets

Table 3 displays the results of APD and TPD of diets studied in this research. All diets with protein (CAS and GLU) showed high values of APD and TPD. The GLU-FFD and GLU-CEL diets showed significantly ($p \leq 0.05$) higher APD and TPD values than the CAS-CEL and experimental diets with IDF (GLU-AGV and GLU-RMD).

Thus, considering N intake and N excretion (Table 2), the absence of DF and the incorporation of CEL to the GLU-based diet promoted higher absorption (93 %) of N intake than AGV and RMD. Rats fed with the CAS-CEL diet showed the lowest N absorption (87 %), which could be related to the high DF intake, causing the lowest APD and TPD, but statistically similar ($P \leq .05$) to GLU-RMD and GLU-AGV diets.

Net Protein Ratio of Diets

Table 3 shows the values of cNPR from the casein and gluten-based diets. The cNPR values were in the range of 1.5 to 4.34.

The group of rats fed with the CAS-based diet showed a higher ($P \leq .05$) value of N use for growth (4.34) than GLU-based diets (1.53-1.78). Rats fed without DF (FFD) or with 5% of DF (SDF or IDF) on GLU-based diets used N absorbed for growing at the same level ($P \leq .05$). As a result, the use of dietary N for growth was more affected by the type of protein (high or low-quality) than by the addition of DF to diets.

Discussion

The WHO recommends a DF intake of 25-35 g/day (García-Montalvo et al., 2018). Diets of 2000-2500 kcal should provide 10-17.5 g of DF/1000 kcal. Similarly, the Academy of Nutrition and Dietetics advises a DF intake of 14 g DF/1000 kcal for children and adults (Dahl and Stewart, 2015).

People from Latin America and the United States consume half or less of the recommended levels of DF. Furthermore, DF intake is still lower in people who eat popular low-carbohydrate diets, like Atkins and South Beach. Only 5% of Americans consume an adequate proportion of DF (Slavin, 2005; Dahl and Stewart, 2015; García-Montalvo et al., 2018).

This situation turns into a concerning issue. For that reason, an increase of DF intake is promoted to help reduce health problems, such as obesity, diabetes, and cardiovascular diseases in developing countries (Slavin, 2005).

Nutrient availability and palatability of a food depend on the concentration and the type of nutrients (Tapsell et al., 2016). Researchers differ on whether DF and nitrogen intake are related to food consumption (Feddern et al., 2008; Pirman et al., 2009; Adams et al., 2018). In this study, feeding was *ad libitum*. Rats ate according to their preferences, and even so, they ate quantities of DF within the recommended range. Groups with SDF ingested slightly more DF than groups fed with IDF. That could be due to SDF producing short-chain fatty acids that stimulate the production of hormones regulating satiation and appetite (Vilcanqui-Pérez and Vílchez-Perales, 2017). Finally, the acceptance of food by rats seems to have been mostly determined by protein type, rather than by the absence of DF or the presence of 5% of DF. The experimental animals consumed more CAS-based food, then GLU-based food and even more than the NFD. Differences could be due to palatability or the type of nutrients intake in each diet.

Protein is associated with functional and tissue protein synthesis, growth, reproduction, maintenance, and repair of organisms. A lack of protein in diets could affect the craving for food and energy (Feddern et al., 2008; Jin et al., 2015). For that reason, rats fed with NFD-CEL could eat lower proportions of food. Despite this, the quantity of calories provided by NFD-CEL was similar to that provided by the other diets. That energy was from carbohydrates and lipids.

The higher nutritional quality and the better acceptance of CAS-CEL triggered higher rat weight. Instead, the lack of protein (NFD-CEL) caused a decrease in rats weight. GLU-based diets prompted an intermediate intake of food, nitrogen, and DF, and low rat weight increase. Null or 5 % DF did not cause a significant effect on rat weight compared with the type of protein. Thus, 5% DF could help to improve the nutritional habits for better health without affecting N intake for growth.

In general, the availability of nutrients from foods to be digested and used by living organisms for growth and maintenance determines their nutritional quality (Feddern et al., 2008, da Silva et al., 2016). Dry matter digestibility (DMD) represents the percentage of dietary nutrients assimilated into the blood capillaries (Goodman, 2010).

The values of DMD reached by the six diets analyzed indicate that all of them can help the digestive system turn nutrients into beneficial substances for nourishment with no trouble. Although IDF triggered a slightly negative effect on DMD compared to SDF, that could be related to the particular action of each type of DF in the digestive system and the slightly higher consumption of IDF than of SDF. IDF accelerates the passage of food through the stomach and intestines, decreasing the digestion of their nutrients, whereas SDF slows down nutrient digestion and absorption in the stomach and intestine (Lattimer and Haub, 2010).

Protein digestibility values reflect the nitrogen absorbed through the digestive tract. APD considers that all fecal nitrogen comes from the diet (Goodman, 2010). Conversely, TPD considers that the intestinal content is composed of dietary protein and endogenous proteins from digestive secretions or epithelial cells. Thus, TPD shows a protein quality value closer to the real value than APD (Darragh and Hodgkinson, 2000; Laleg et al., 2019).

To calculate TPD, it is necessary to deduct the metabolic fecal nitrogen by subtracting the fecal nitrogen excretion by a group of rats fed with a nitrogen-free diet (Laleg et al., 2019). Therefore, TPD values are higher than those of APD. The results of protein digestibility (higher than 89.7 %) from the experimental diets indicate efficient nitrogen absorption. So, the diets used in this research help the digestive system turn protein into beneficial substances for nutrition and body-maintenance. Conversely, researchers have noticed that substantially high quantities (≥10 %) of total DF (SDF and IDF) cause a substantial decrease in protein digestibility. That suggests an interaction between DF and dietary protein, and that a high DF intake results in the microflora reaching its fermentative capacity and/or in an enlargement of the digestive tract, causing lower nutrient digestibility (Jørgensen et al., 2003; Falcón-Villa et al., 2014).

DF properties can affect the bioavailability of food nutrients (Adams et al., 2018). Both SDF decreased the protein digestibility of the gluten-based diets compared to IDF or the absence of fiber, as previously observed (Slavin, 2005; Pirman et al., 2009; da Silva et al., 2016), despite the slightly lower SDF ingested than IDF of gluten-based diets.

Fermentability and viscosity depend on the size and chemical composition of each DF. These properties affect the way each DF delays or avoids the digestion of nutrients. AGV and RMD are low-molecular-weight SDF, highly fermentable, but with lower viscosity than other SDF. In contrast, CEL is a non-fermentable, non-viscous IDF with high molecular weight (Vilcanqui-Pérez and Vílchez-Perales, 2017).

SDF dissolves in water and triggers viscous gels with a tridimensional structure that avoids physical contact between digestive enzymes and nutrients, thus decreasing nutrient digestion (Vilcanqui-Pérez and Vílchez-Perales, 2017). Fermentation stimulates the growth of microbes, and viscosity increases fecal nitrogen excretion triggered by the high bacterial activity in the large intestine (Adams et al., 2018). Then, viscosity rises to form a barrier in the lumen, avoiding protein digestion (Wong and Cheung, 2003). Also, viscosity slows gastric emptying and extends the feeling of fullness, delaying nutrient absorption and reducing the degree of protein metabolism in the small intestine (Slavin, 2005; Adams et al., 2018). On the other hand, IDF does not form a gel due to its limited fermentation and water insolubility (Verma and Banerjee, 2010). However, IDF is related to higher fecal volume and mass and to a reduction of intestinal transit time (Vilcanqui-Pérez and Vílchez-Perales, 2017), which could allow higher protein absorption than SDF.

After absorption of nutrients, e.g., protein, a significant factor is their utilization for maintenance and growth. The control CAS-CEL diet displayed a better capacity to supply protein for easy absorption for weight increase and maintenance. That is because casein contains high-quality essential amino acids, resulting in a high-quality protein (Laleg et al., 2019).

In contrast, the gluten-based diet showed lower cNPR than the casein-based food, and the addition of 5% SDF and IDF concerning the absence of DF did not affect the capacity of the protein for maintenance and growth. Cereal proteins, such as gluten, are deficient in some essential amino acids such as lysine and have poor performance in promoting rat weight gain . The protein network of gluten pasta is formed by multiple covalent bonds, essentially disulfide. Indeed, gluten protein has a significantly lower nutritional quality for rat growth than casein and legumes (Laleg et al., 2019).

In that way, 5 % of AGV, RMD, and CEL didn´t affect protein digestibility, and the use of N for growth on diets formulated with 12.63 g of DF/1,000 kcal. In addition, AGV is a good alternative as a DF source to increase benefits to Agave industries.

Conclusions

People around the world eat gluten-based food on a daily basis. Also, most of these people consume refined and sugary foodstuffs, which eventually promote the emergence of diseases. Formulating diets including DF and protein from different food groups is relevant to acquiring adequate and complete amino acids for body maintenance and proper weight increase. Thus, food formulated with 13 g DF/1,000 kcal could help maintain an adequate level of nutrition to promote good health, even in low-quality protein diets. So, cellulose, resistant maltodextrin, and agave inulin are suitable sources of DF, and they could be used in combination to provide the benefits of both types of DF. Finally, *Agave tequilana* Weber var. Azul fructan (Agavin) is a good dietary DF source, representing an economic benefit and development option for the agave industry.

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