MARIA SAMIRES MARTINS CASTRO

DIFFERENT SOURCES OF NON-FIBROUS CARBOHYDRATES FOR LACTATING GOATS

Thesis submitted to the Animal Science Graduate Program of the Universidade Federal de Viçosa, in partial fulfillment of the requirements for the degree of *Doctor Scientiae.*

Advisor: Cristina Mattos Veloso

Co-Advisor: Ricardo Augusto Mendonça Vieira

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Assent:

Documento assinado digitalmente
MARIA SAMIRES MARTINS CASTRO
Data: 27/09/2024 09:12:54-0300
Verifique em https://validar.iti.gov.br

Maria Samires Martins Castro
Author

Documento assinado digitalmente
CRISTINA MATTOS VELOSO
Data: 27/09/2024 11:10:36-0300
Verifique em https://validar.iti.gov.br

Cristina Mattos Veloso
Adviser

BIOGRAPHY

MARIA SAMIRES MARTINS CASTRO, daughter of Maria Socorro Martins Castro and Carlos Augusto Sousa Castro, was born in Itapajé, Ceará, on December 11th, 1995.

In July of 2013, she began the undergraduation in Animal Science course at Universidade Estadual Vale do Acaraú (UVA), in the city of Sobral, Ceará, under the guidance of Professor Dra. Angela Maria de Vasconcelos. She finished in August of 2018.

In August of 2018, she began her Master's degree in Animal Science Postgraduate Program at UVA, under the guidance of Professor Dra. Angela Maria de Vasconcelos, and co-supervision of Professor Delano de Sousa Oliveira, obtaining the title of Master in July of 2020.

In August 2020, she began the Doctorate in Animal Science Postgraduate Program at the Universidade Federal de Viçosa (UFV), under the advisoring of Professor Cristina Mattos Veloso, and co-supervision of Professor Ricardo Augusto Mendonça Vieira, submitting to the Thesis defense on the day August, 5th of 2024.

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ABSTRACT

CASTRO, Maria Samires Martins. *D.Sc.*, Universidade Federal de Viçosa, August, 2024. **Different sources of non-fibrous carbohydrates for lactating goats.** Adviser: Cristina Mattos Veloso. Co-Adviser: Ricardo Augusto Mendonça Vieira.

Carbohydrates are the most abundant biomolecules in nature and account for 70 to 80% of ruminant diets. Among the classifications, non-fibrous carbohydrates have been commonly used to increase milk production. Thus, the objective of this study was to evaluate the effects of the use of non-fibrous carbohydrates (NFC), such as cornmeal, pre-gelatinized corn, citrus pulp and sucrose, on nutrient intake, digestibility, milk yield and composition in lactating dairy goats. Twenty-nine primiparous (11) and multiparous (18) lactating Saanen and Alpine goats (2.8 ± 0.27 kg.d-1 milk; 55.4 ± 1.8 kg body weight) were used in this study. The goats were distributed in eight pens with four animals each, with the pen being the experimental unit and the animals being the observational units, and in four diets in a 4×4 balanced double Latin square design, with four periods of 21 days each. During the first period of the experiment, three goats stopped producing milk and were removed from the experiment. The treatments were corn meal, pre-gelatinized corn, citrus pulp and sucrose, with the roughage based on corn silage, and the roughage:concentrate ratio 60:40. The diets were formulated to meet the nutritional requirements of lactating goats and were offered in four daily meals, in the same physical form. Voluntary nutrient intake, digestibility, production weighing and samples for analysis of milk composition were performed during the seven days of each collection period. We did not consider the effect of body weight, due to the short evaluation periods present in the Latin square design, which tend to hinder the variation of weight gain, not correcting errors in ruminal filling. Goats fed corn meal showed higher lignin intake and digestibility of dry matter, organic matter and fiber, and lower intake when sucrose was used. Citrus pulp also stood out for its fiber digestibility and influenced some milk components, such as fat, protein, total solids, milk fat-corrected and net lactation energy. However, milk composition showed low values when the goats were fed sucrose. Regarding the period, only fat, milk fat-corrected and net lactation energy were influenced. Regarding ingestive behavior, there was an influence of the diet for intake, being greater when the goats received corn meal and smaller when they received pre-gelatinized corn. Regarding idleness, we observed the opposite effect, being greater in the pre-gelatinized corn diet and smaller with corn meal in the diet. We suggest that citrus pulp can be used as a concentrated feed for dairy goats, without compromising nutrient intake, digestibility, milk yield and composition. However, diets with sucrose affect intake, digestibility, milk production and composition.

Keywords: Citrus pulp. Digestibility. Milk production. Sucrose.

RESUMO

CASTRO, Maria Samires Martins. *D.Sc.*, Universidade Federal de Viçosa, agosto de 2024. **Diferentes fontes de carboidratos não fibrosos em dietas para cabras lactantes.** Orientadora: Cristina Mattos Veloso. Coorientador: Ricardo Augusto Mendonça Vieira.

Os carboidratos são as biomoléculas mais abundantes na natureza e correspondem de 70 a 80% das dietas dos ruminantes. Dentre as classificações, os carboidratos não fibrosos vêm sendo comumente utilizados para aumentar a produção leiteira. Assim, objetivou-se avaliar os efeitos do uso de carboidratos não fibrosos (CNF), como fubá, milho pré-gelatinizado, polpa cítrica e sacarose, sobre o consumo de nutrientes, digestibilidade, produção e composição do leite em cabras leiteiras em lactação. Vinte e nove cabras primíparas (11) e multíparas (18), lactantes da raça Saanen e Alpina (2,8 ± 0,27 kg.d^{-1} leite; 55,4 ± 1,8 kg de peso corporal) foram utilizadas neste estudo. As cabras foram distribuídas em oito baias com quatro animais cada, sendo a baia a unidade experimental e os animais as unidades observacionais e ainda em quatro dietas em delineamento quadrado latino duplo balanceado 4×4, com quatro períodos de 21 dias cada. Durante o primeiro período do experimento, três cabras cessaram a produção de leite e foram retiradas do experimento. Os tratamentos foram fubá de milho, milho pré-gelatinizado, polpa cítrica e sacarose, sendo o volumoso a base de silagem de milho, e a proporção volumoso:concentrado 60:40. As dietas foram formuladas para atender as exigências nutricionais de cabras em lactação e foram oferecidas em quatro refeições diárias, na mesma forma física. O consumo voluntário de nutrientes, digestibilidade, pesagem da produção e amostras para análise de composição do leite foram realizados durante os sete dias de cada período de coleta. Não consideramos o efeito do peso corporal, devido aos curtos períodos de avaliação, presentes no desenho do quadrado latino, que tendem a dificultar a variação do ganho de peso, não corrigindo erros de enchimento ruminal. Cabras alimentadas com fubá de milho apresentaram maior consumo de lignina e digestibilidade da matéria seca, matéria orgânica e fibra, e menor consumo quando a sacarose foi utilizada. A polpa cítrica também se destacou pela digestibilidade da fibra, e influenciou em alguns componentes do leite, tais como a gordura, proteína, sólidos totais, leite corrigido para gordura e energia líquida de lactação, no entanto a composição do leite apresentou baixo valores, quando as cabras foram alimentadas com sacarose. Quanto ao período, apenas gordura, leite corrigido para gordura e energia líquida de lactação foram influenciados. Quanto ao comportamento ingestivo, houve influência da dieta para consumo, sendo maior quando as cabras receberam fubá de milho e menor quando receberam milho pré-gelatinizado. Em relação ao ócio, nós observamos efeito contrário, sendo maior na dieta de milho pré-gelatinizado e menor com fubá na dieta. Sugerimos que a polpa cítrica pode ser utilizada como ração concentrada para cabras leiteiras, sem comprometer o consumo de nutrientes, digestibilidade, produção e composição do leite. Entretanto, dietas com sacarose afetam consumo, digestibilidade, produção e composição do leite.

Palavras-chave: Açúcar. Digestibilidade. Polpa cítrica. Produção de leite.

LIST OF TABLES

LIST OF ACRONYMS AND ABBREVIATIONS

ADL - Acid detergent lignin

ADLi - Acid detergent lignin intake

AFRC - Agricultural and Food Research Council

ANL - Animal Nutrition Laboratory

AOAC - Association of Official Analytical Chemists

BW - Body weight

$\mathbf{C_{ADL}}$ - Concentration of acid detergent lignin

$\mathbf{C_{ash}}$ - Concentration of ash

CEUAP - Ethics Comission on the Use of Farm Animals

$\mathbf{C_{CF}}$ – Concentration of crude fat

CF - Crude fat

CHO - Carbohydrates

$\mathbf{C_{i}}$ - Concentration of nutrients in the intake

$\mathbf{C_{CP}}$ - Concentration of crude protein

$\mathbf{C_{NDF}}$ - Concentration of neutral detergent fiber

$\mathbf{C_{OM}}$ - Concentration of organic matter

CP - Crude protein

DCP - Dried citrus pulp

DDGS - Dried distillers grains with solubles

DM - Dry matter

DMDC - Dry matter digestibility coefficient

DMI - Dry matter intake

EOC - Exhausted olive cake

ESALQ/USP - Escola Superior de Agricultura Luiz de Queiroz da Universidade de São Paulo

FA - Fatty acid

FC - Fibrous carbohydrates

FCM - Fat corrected for milk

$\bar{F}^{S}_{ADLi}$ - Average daily intake rate of acid detergent lignin

$\bar{F}^{S}_{ashi}$ - Average daily intake rate of ash

$\bar{F}^{S}_{CFi}$ - Average daily intake rate of crude fat

$\bar{F}^{S}_{CPi}$ - Average daily intake rate of crude protein

$\bar{F}^{S}_{i}$- Average daily intake rate of dry matter

$\bar{F}^{S}_{NDFi}$ - Average daily intake rate of neutral detergent fiber in organic matter

$\bar{F}^{S}_{OMi}$ - Average daily intake rate of organic matter

HS - High sugar

INCT- CA - National Institute of Science and Technology - Animal Science

ISO - International Organization for Standardization

LS - Lower sugar

LTDA - Limited company

MG – Minas Gerais

MM - Mineral matter

MY - Milk yield

MYp - Previous milk yield

NC/USA - North Carolina/United States of America

NDFDC - Neutral detergent fiber digestibility coefficient

aNDFom - Amylase-treated neutral detergent fiber organic matter

NEFA - Non-esterified fatty acids

NEL - Net energy of lactation

NFC - Non-fibrous carbohydrates

NRC - National Research Council

NSC - Non-structural carbohydrates

OM - Organic matter

OMDC - Organic matter digestibility coefficient

PGC - Pre-gelatinized corn

PR - Paraná

SAS - Statistical Analysis System

SCC - Somatic cell count

SME - Standard mean error

SP - São Paulo

TS - Total solids

TSNF - Total solids non-fat

UENF – Universidade Estadual do Norte Fluminense

UFV - Universidade Federal de Viçosa

SUMMARY

1. GENERAL INTRODUCTION

Carbohydrates constitute the main source of energy in the diet of dairy ruminants and generally comprise 60 to 70 percent of the diet. Furthermore, nutritionally, they are partitioned according to their digestion characteristics in the animal. It is important to highlight that fibrous carbohydrates (FC) have two main functions: to stimulate rumination and insalivation and to prevent too rapid passage and loss of nutrients. While non-fibrous carbohydrates (NFC) generally ferment faster and more extensively than insoluble fiber, and provide a valuable source of energy and carbon for microbial fermentation (Van Soest, Robertson and Lewis, 1991; Hall and Mertens, 2017; NASEM, 2021).

The knowledge of the types of carbohydrates in diets for dairy ruminants is essential, since there are important relationships between carbohydrate fermentation rates and microbial efficiencies, per unit of feed digested in the rumen (Van Soest, Robertson and Lewis, 1991; Hall and Mertens, 2017; Oba and Kammes-Main, 2023). For example, pectin invariably is the most rapidly degraded complex carbohydrate, whereas starches are quite variable according to source. Furthermore, pectin fermentation stands out not only due to the lack of lactic acid production, but also due to the structure of galacturonic acid that provides potential, through cation exchange and binding of metal ions. Sucrose and starch are NFCs that also differ from each other in terms of ruminal fermentation characteristics. Sucrose disappears quickly in the rumen up to 738%/h, while starch ferments more slowly (Van Soest, Robertson and Lewis, 1991; Hall and Zanton, 2022).

In dairy ruminant production systems, diets using corn in the feeds prevail. Although 75% of corn's energy value is derived from starch, it is surrounded by a protein matrix in the endosperm that influences digestion by microorganisms. Therefore, some processes, such as flocculation, steam lamination and pelletization where the grains are hydrated, heated and pressed, are techniques that have been used to improve the accessibility of starch for enzymatic digestion, mainly through hydration or gelatinization (Ferraretto, Crump and Shaver, 2013; Rahimi et al. 2020; Rahpeyma et al. 2024).

However, despite several studies using alternative sources to corn grain starch to dairy cows, studies with dairy goats are still scarce. Some research evaluating non-fibrous carbohydrates in dairy goats are reported by Lunesu et al. (2021), who evaluated different performances during lactation because of a varied starch concentration in the diets. Regarding pectin, Marcos et al. (2020) evaluated the digestibility of diets composed of dry citrus pulp. Sari, Naserian and Validazeh (2009), also verified pectin infusion and found no significant effects on dry matter and neutral detergent fiber digestibility. Guzmán et al. (2020), found no significant differences on average milk production and composition, when dehydrated orange pulp was used in the diet of dairy does.

Thus, the objective of this review is to describe the types of carbohydrates, emphasizing non-fibrous carbohydrates in the diet of dairy ruminants on the nutrients intake and digestibility, and yield and composition of milk.

2. LITERATURE REVIEW

2.1 INTRODUCTION TO NON-FIBROUS CARBOHYDRATES

Carbohydrates (CHO) in vegetables derive from the fixation of atmospheric CO_2, through the Calvin process, exerted by autotrophic organisms, where light energy is converted into chemical energy during photosynthesis in chloroplasts. These living organisms are responsible for the most abundant compounds in the biosphere (Cabral et al., 2002).

Since the 1800s, chemists have attempted to characterize nutritionally relevant fractions in feed. Thus, given the understanding of CHO, specifically non fiber carbohydrates (NFC), the applicability of analyzes for formulating diets advances and changes the types of errors that affected the measurements. However ionic, physical and covalent relationships of molecules in plant material that influence the solubility of CHO for separation may not remain uniformly in plant materials (Hall, 2003).

In practice, CHO fractions are defined by chemical or enzymatic methods. Its partition depends on differences in solubility and enzymatic specificity. As for common use, NFC corresponds to products such as organic acids, monosaccharides, oligosaccharides, fructans, starch, pectic substances, (1 → 3) (1 → 4)-β-glucans, and other CHO. In the NFC group there is also a subgroup, called non-structural carbohydrates (NSC), which are deprived of CHO with cellular content, including monosaccharides, oligosaccharides, fructans and starch. Among NFC, monosaccharides, maltose, and starch are digestible by mammalian enzymes, and sucrose and lactose can be digested, but with some variation in this capacity between species and individuals (Hall, 2003).

CHO are chemically defined as polyhydroxy ketones (ketoses) or polyhydroxy aldehydes (aldoses) (Nelson and Cox, 2014). They are a predominant source of energy, generally constituting around 70 to 80% of the ruminant diet (Bomfim, Teixeira and Oliveira, 2023; Oba and Kammes-Main, 2023). From a nutritional point of view, they can be classified into fibrous CHO (FC), such as cellulose and hemicellulose, which together with lignin make up the cell wall and are slowly digested, with a variable nutritional availability and take up space in the gastrointestinal tract or NFC. These are is characterized by the fractions degraded more quickly in the digestive tract (starch, sucrose and pectin) of ruminants (Cabral et al., 2002; Ravelo et al., 2022).

The NFC diversity may introduce variation in digestion end products in terms of microbial mass and fermentation acids, as they generally ferment faster than insoluble fiber and provide a valuable source of energy and carbon for microbial fermentation. Moreover, when different sources of CHO are supplied, varying amounts of energy (ATP) are observed for the microbes, for example, the formation of acetate results in greater microbial ATP gain than propionate. However, excessive feeding of fermentable CHO can cause rumen acidosis and digestive disorders (Gao and Oba, 2016; Hall and Mertens, 2017; Pfau et al., 2021).

Taghipoor, Delattre, and Giver-Reverdin (2020), when evaluating a new modeling approach to quantify dairy response goats on a highly concentrated diet demonstrated high variability among goats in response to the new diet, highlighting in particular their daily and general strategies for buffering the effect of the change in diet. Furthermore, they emphasized that goats have a higher pH (+0.4 pH) than that of cattle when fed diets with a similar percentage of neutral detergent fiber (NDF) or concentrate, which implies that the threshold below which animals develop acidosis may be different for large and small ruminants.

2.1.1 Starch

One of the main sources of NFC supplied in the diets of dairy animals is corn (*Zea mays*), although its ruminal starch degradation rate is considered low, around 15%/h. About 75% of the energy value of corn grain is derived from starch. However, the basic morphology of the corn kernel consists of pericarp, germ and endosperm, with the germ and endosperm being surrounded by the pericarp, which is largely resistant to microbial fixation. Starch processing methods result in higher degradation rates (Ferraretto, Crump, Shaver, 2013 and Hanlon et al., 2023).

Starch is a non-structural polysaccharide with an energy reserve function, chemically formed by two glucose polymers, amylose and amylopectin. Since the percentage of amylose and amylopectin varies with the botanical origin of the starch. In most species, starch is composed of 30% amylose and 70% amylopectin. In addition to amylose and amylopectin, starch granules are composed of lipids, proteins and minerals, which composition depends on the species and the part of the plant in which it is found (Antunes, Rodriguez and Saliba, 2011).

2.1.2 Sugar

Sugars can be defined as monosaccharides (simple sugars), disaccharides and oligosaccharides, being separated from polysaccharides due to their solubility in 80% ethanol. Glucose and fructose are the most common simple sugars found in plants. And the most abundant disaccharide in vegetables is sucrose (glucose + fructose). Lactose (glucose + galactose) is the disaccharide found in milk. There are also oligosaccharides, which are monosaccharides two to 20 units long, including stachyose and raffinose. However, plants have few oligosaccharides, and with the exception of these, sugars are digestible by mammalian enzymes (Hall, 2002).

Among NFC, sugars are soluble carbohydrates, as they solubilize in the aqueous environment of the cell, normally in the cytoplasm. They make up part of the NFC that do not include starch. Unlike starch, processing methods are not aimed to soluble CHO, since they are already available to microorganisms and enzymes, and are generally soluble in water or ethanol. They can be supplied directly as sugar (sucrose and lactose) or incorporated through by-products, such as molasses and whey in ruminant diets to increase sugar concentration (Ravelo et al., 2022).

There are different sources of sugar. Among the varieties, sugar beet (*Beta vulgaris*) stands out, with sucrose extracted from the deep root, while in sugar cane it derives from the medullary tissues of the trunk. Despite the similarities, they have different organoleptic characteristics. Molasses is another source of sugar that has been used in animal nutrition. It consists of a thick liquid, brown in color and syrupy in consistency, which is the residue remaining after sugar extraction when it is no longer possible to obtain sucrose (Mordenti et al., 2021).

Regarding the use of molasses in ruminant diets, Mordenti et al. (2021) point out some advantages and disadvantages. For example, one of the effects that occur with the inclusion of molasses in ruminant diets is the increase in dry matter intake, which is even greater when referring to pasture animals (cattle, buffaloes, sheep and goats) if fed poor quality forage. Under these conditions, molasses has stimulating effect on the digestive activity of the rumen microbiota, thus improving both the digestibility of coarse quality forage and dry matter intake. However, more attention should be given to anion-cation balanced diet, when molasses is used, because it can be altered due to the greater potassium content.

Sugars such as sucrose and fructose are another common source of NFC, which are rapidly fermented in the rumen, with degradation rates of 40 to 60%/h (Palmonari et al., 2020; Hanlon et al., 2023). This process occurs partly because sugar provides approximately 10% less carbon than starch per unit mass, due to fewer glycosidic bonds (Oba and Kammes-Main, 2023). Furthermore, diets with sugars tend to influence DMI, milk fat production and yield, improvements in nitrogen production and utilization and higher butyrate concentration (Ravelo et al., 2022). Although sugar ferments rapidly in the rumen, often increased DMI of dairy animals may be associated with the preference for sweet taste or decreased propionate flow to the liver (Oba and Kammes-Main, 2023).

Penner and Oba (2009), when evaluating the increasing dietary sugar concentration in postpartum dairy cows, observed that feeding higher sugar (HS = 8.4%) increased DMI during the first four weeks of lactation by 1.1 kg/d, when compared with cows fed lower sugar (LS= 4.7%).

2.1.3 Pectins

Pectins are complex polysaccharides that include galacturonic acid, arabinose, galactose, xylose, rhamnose, glucuronic acid and fucose. They are therefore included in NFC. Pectin is located in the cell wall, and considered a structural CHO, but is soluble in a neutral detergent solution and is quickly digestible by rumen microorganisms. Furthermore, the fermentation of pectin, a component resulting from dried citrus and beet pulp, results in a high proportion of acetate to propionate, but little or no lactate (Villalba, Ates and MacAdam, 2021).

The use of agricultural by-products to feed ruminants is associated with climate change that has led to a shortage of animal feed in many parts of the world. In addition to being a viable option for the sustainability of dairy production systems, reducing the dietary proportion of human edible products (López et al., 2014; Dadashi et al., 2023). Among the by-products, orange pulp stands out, which results from the production of orange juice, composed of membranes, peels, residual pulp and seeds. Its use is beneficial due to recycling, reduced environmental pollution and reduced costs. And it has been used as a source of metabolizable energy in ruminant feeding, in addition to including abundant levels of pectin (223 g/kg dry matter, DM) which is fermented in the rumen without affecting pH (Münnich et al., 2017; Villalba, Ates and MacAdam, 2021; Dadashi et al., 2023).

Commercially available pectin comes mainly from three main residues: sugar beet pulp, citrus peel and apple pomace. As for citrus fruits, they represent almost 98% of the total number of industrialized crops, with oranges (*Citrus sinensis*) representing around 82% of the total citrus fruits produced, followed by tangerines (*Citrus reticulate*) (7.26%), lemon (*Citrus limon*)/lime (*Citrus aurantiifolia*) (9.74%) and grapefruit (*Citrus paradisi*) (3.00%) (Suri, Singh and Nema, 2022; Roman-Benn et al., 2023). Regarding global citrus fruit processing, Brazil is the leading country with 12.11 million tons of citrus fruits. It is important to highlight that citrus fruits used for industrial processing generate more than 10 million tons of waste annually. In general, citrus fruits originating from the Rutaceae family are one of the fruits extensively cultivated throughout the world.

2.2 INTAKE AND DIGESTIBILITY OF NON-FIBROUS CARBOHYDRATES IN GOATS AND DAIRY COWS

2.2.1 Starch

The digestibility of NFC can be affected in several ways. In the case of starch digestibility, generally it is affected by characteristics of the granules, the type of grain due to differences in genotype, type of endosperm, the size, crystallinity and polymerization degree, non-starch components, their interactions with starch and their amylose:amylopectin ratio. Furthermore, the processing method and preservation method, and the extent of starch digestion in the rumen can affect dry matter intake (Wang, Wang and Tan, 2016; Kokic et al., 2022; Oba and Kammes-Main 2023).

Therefore, processing techniques are necessary due to the whole grain, which has an intact pericarp and is highly resistant to digestion by ruminants due to the inability of rumen bacteria to attach to their surface. Therefore, it may appear 30% of whole grain in feces of cattle fed whole grain diets. In this way, the cereal grain is processed using different combinations of heat, humidity, time and mechanical action. These processing treatments allow bacteria to attach to the starch granules, thus increasing their digestibility. Furthermore, processing breaks the structure of the endosperm, breaks the protein matrix that surrounds the starch granules in the endosperm and gelatinizes the starch granules, making it more available to both ruminal microbial and pancreatic enzymes (Kokic et al., 2022).

To improve starch availability, according to Kokic et al. (2022) and Rahimi et al. (2020), some techniques are used, such as grinding, dry rolling, steam rolling and steam peeling, steam-flaking, cover pelletizing, extrusion and expansion, also including gelatinization, which involves the dissolution of hydrogen bonds among and within starch molecules to open the molecules until hydration and enzymatic hydrolysis. In this way, the crystalline structure of the granules must be broken to complete enzymatic hydrolysis of starch. Gelatinization is normally carried out with heating (90 to 100 °C) with water or tampon, or, alternatively, with the use of a base (e.g. potassium hydroxide) followed by neutralization (Hall, 2007).

Starch gelatinization is a process that involves heating in the presence of excess water, thus undergoing an order-disorder phase transition in a temperature range characteristic of the starch source. Furthermore, the transition phase is associated with diffusion of water into the granule, absorption of water by the amorphous bottom region, hydration and radial swelling of starch granules, loss of optical birefringence, heat absorption, loss of crystalline order, uncoiling and dissociation of double helices (in the crystalline regions) and leaching of amylose (Hoover, 2001).

The process of intestinal starch assimilation begins in the lumen of the small intestine with the secretion and action of pancreatic α-amylase. α-Amylase is synthesized in pancreatic acinar cells. When synthesized, α-amylase and other enzymes are packaged in zymogen granules and stored until a stimulus signals the cell to initiate exocytosis event to release enzymes into the duodenum lumen. In addition to the pancreatic enzyme, mucosal enzymes also act on starch digestion, however the mechanism in ruminants is not yet well understood, regarding proteins that have carbohydrase activity in non-ruminants (Harmon, Yamka and Elam, 2004).

Xu et al. (2009) reported that low starch digestibility in goats may also be associated with insufficient enzyme activity. Among them, those of pancreatic exocrine secretion stand out, such as α-amylase, trypsin, chymotrypsin and lipase, which are fundamental in ruminant digestion.

2.2.2 Citrus pulp and sugar

On the apparent digestibility of nutrients in dairy goat diets composed of agro-industrial by-products, such as dehydrated distiller's grains with solubles (DDGS) and dried citrus fruits pulp (DCP), Marcos et al. (2020) found no differences ($P \geq 0.15$) between the diets on the apparent digestibility of nutrients, with the exception of fat digestibility, since DDGS fat is highly digestible. Sari, Naserian and Validazeh (2009), when evaluating the effects of infusion of 0, 40, 80 or 120 g/d of citrus pectin in the abomasum of Saanen dairy goats on digestion, found an insignificant decrease in DM and NDF total apparent digestibilities.

Hall and Zanton (2022) evaluated the replacement of cane molasses with corn grain in lactating cows, and observed that there was apparent digestibilities of the total tract of DM, OM and NDF were all affected by molasses, with quadratic patterns for all digestibilities (P≤0.05), with the lowest value always in the molasses treatment at 5.25%.

2.3 EFFECTS OF NON-FIBROUS CARBOHYDRATES ON THE PRODUCTION AND QUALITY OF MILK IN GOATS AND DAIRY COWS

2.3.1 Starch and Citrus pulp

Tsiplakou et al. (2017), evaluating the response of goats to different proportions of starch/NDF concentrates in milk chemical composition, found no difference in milk yield and composition, between the two dietary treatments, in which different proportions of starch/NDF, were 1.2 and 0.3 for high starch (HS) and low starch (LS), respectively. Zang et al. (2021), in a study that evaluated the level of starch in the diet and methionine, lysine and histidine protected in the rumen, in the milk production in dairy cows fed diets low in metabolizable proteins, observed that diets with high starch content (30% ground corn) and high starch +AA increased milk production (37.9 vs. 40.1 kg/d) and milk protein. Milk (1.07 vs. 1.16 kg/d) and decreased dry matter intake (25.9 vs. 25.2 kg/d).

A study carried out by Guzmán et al. (2020), using dehydrated orange pulp in dairy goats diet on the effects of milk yield and composition, found no difference in the average daily milk production, 1.66 L. Marcos et al. (2020), evaluated the effects of agro-industrial byproducts supplementation on dairy goat's milk characteristics and concluded that a mixture of DDGS corn, DCP and exhausted olive cake (EOC) tends to result in greater production of milk fat and protein, as well as a more unsaturated fatty acid (FA) milk profile. López et al. (2014), when evaluating the use of citrus pulp or soybeans hulls to replace corn grain on milk performance in lactating Murciano-Granadina goats, also did not observe differences in milk production (1.72 kg/d).

3. OBJECTIVES

The objectives with this work were to evaluate nutrient intake in dairy goats fed different types of non-fibrous carbohydrates; check digestibility when fed with different carbohydrates; investigate milk production and composition on such diets; and analyze the ingestive behavior of dairy goats.

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Different sources of non-fibrous carbohydrates for lactating does

Maria Samires Martins Castro[a*], Marcelo Teixeira Rodrigues[a], Ricardo Augusto Mendonça Vieira[b], Cristina Mattos Veloso[a]

[a]Department of Animal Science, Universidade Federal de Viçosa, Viçosa, Minas Gerais 36570-900, Brazil

[b] Animal Science Laboratory, Universidade Estadual do Norte Fluminense, Av. Alberto Lamego 2000, Campos dos Goytacazes, RJ, CEP 28013-602, Brazil

[*]Corresponding author.

E-mail address: samireszootecnia@gmail.com

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CHAPTER 2

DIFFERENT SOURCES OF NON-FIBROUS CARBOHYDRATES FOR LACTATING DOES

ABSTRACT: There is little information on the use of non-fibrous carbohydrates in dairy goat diets. The aim of this study was to investigate the effects of the use of non-fibrous carbohydrates (NFC), such as cornmeal, pregelatinized corn, citrus pulp and sucrose on nutrient intake, digestibility, milk production and composition in lactating does. Twenty-nine primiparous (11) and multiparous (18) lactating Saanen and Alpine goats (2.8 ± 0.27 days in milk; 55.4 ± 1.8 kg body weight) were used in this study. The goats were distributed into four diets in a 4×4 Latin square design, with periods of 21 days. During the first period of the experiment, three goats ceased milk production and were removed from the experiment. The treatments were cornmeal, pre-gelatinized corn, citrus pulp and sucrose, offered in the same physical form, as a total mixed ration, with the bulky feed consisting of corn silage, in a roughage:concentrate ratio of 40:60. We did not consider the effect of body weight, due to the short evaluation periods, present in the Latin square design, which tend to hinder the variation of weight gain, not correcting ruminal filling errors. Lignin intake was higher with the corn meal diet and lower when sugar was used. Citrus pulp stands out for its fiber digestibility. Regarding milk yield and composition, we observed that the citrus pulp diet positively influenced the production of fat, protein, total solids, fat-corrected milk and net lactation energy, while negatively for the diet composed of sucrose. Regarding the period, there was an effect only for fat, fat-corrected milk and net lactation energy, being higher in period one and lower in period four. In terms of ingestive behavior, intake was higher when the goats received corn meal and lower when they received pre-gelatinized corn. Regarding idleness, we observed the opposite, being higher in the pre-gelatinized corn diet and lower with corn meal. We suggest that citrus pulp can be used in dairy goat feed without compromising nutrient intake, digestibility, milk yield and composition. However, a diet with sucrose affects intake, digestibility, milk yield and composition parameters.

Keywords: Corn meal. Nutrients. Pulp citrus. Sucrose.

CAPÍTULO 2

DIFERENTES FONTES DE CARBOIDRATOS NÃO FIBROSOS PARA CABRAS LACTANTES

RESUMO: Existem poucas informações sobre o uso de carboidratos não fibrosos na alimentação de cabras leiteiras. O objetivo deste estudo foi investigar os efeitos do uso de carboidratos não fibrosos (CNF), como fubá, milho pré-gelatinizado, polpa cítrica e sacarose sobre o consumo de nutrientes, digestibilidade, produção e composição do leite em cabras leiteiras em lactação. Vinte e nove cabras primíparas (11) e multíparas (18), lactantes, Saanen e Alpine (2,8 $\pm$ 0,27 dias em leite; 55,4 $\pm$ 1,8 kg de peso corporal) foram utilizadas neste estudo. As cabras foram distribuídas em quatro dietas em delineamento em quadrado latino 4×4, com períodos de 21 dias. Durante o primeiro período do experimento, três cabras cessaram a produção de leite e foram retiradas do experimento. Os tratamentos foram fubá, milho pré-gelatinizado, polpa cítrica e sacarose, ofertadas na mesma forma física, como ração total mista, sendo o volumoso composto por silagem de milho, numa relação volumoso:concentrado 40:60. Não consideramos o efeito do peso corporal, devido aos curtos períodos de avaliação, presentes no desenho do quadrado latino, que tendem a dificultar a variação do ganho de peso, não corrigindo erros de enchimento ruminal. O consumo de lignina foi maior com a dieta fubá de milho e menor quando utilizado o açúcar. A polpa cítrica destaca-se pela digestibilidade da fibra. Em relação à produção e composição do leite, observamos que a dieta de polpa cítrica influenciou positivamente a produção de gordura, proteína, sólidos totais, leite corrigido para gordura e energia líquida de lactação, enquanto negativamente para a dieta composta por sacarose. Quanto ao período, houve efeito apenas para gordura, leite corrigido para gordura e energia líquida de lactação, sendo maiores no período um e menores no período quatro. No comportamento ingestivo, o consumo foi maior quando as cabras receberam fubá e menor quando receberam milho pré-gelatinizado. Em relação ao ócio, observamos o contrário, sendo maior na dieta de milho pré-gelatinizado e menor com o fubá de milho. Sugerimos que a polpa cítrica pode ser utilizada na alimentação de cabras leiteiras, sem comprometer o consumo de nutrientes, a digestibilidade, o rendimento e a composição do leite. Entretanto a dieta com sacarose afeta parâmetros de consumo, digestibilidade, produção e composição do leite.

Palavras-chave: Fubá de milho. Nutrientes. Polpa cítrica. Sacarose.

1. Introduction

Carbohydrates constitute the main source of energy in the diet of dairy ruminants and generally comprise 60 to 70 percent of the diet. Furthermore, they are partitioned nutritionally into fibrous (FC) and non-fibrous carbohydrates (NFC). The NFC generally ferment faster and more extensively than insoluble fiber, which provide a valuable source of energy and carbon for microbial fermentation (Van Soest, Robertson and Lewis, 1991; Hall and Mertens, 2017; NASEM, 2021).

Different sources of carbohydrates have been added to diets of dairy animals, progressively becoming important substitutes for cereal grains. Among carbohydrates, the sugars sucrose and lactose stand out, which have the potential to increase dry matter intake, and improve the output of milk components, whereas diets rich in pectin result in higher milk fat contents (Van Soest, Robertson and Lewis, 1991; Hall and Mertens, 2017; Guzmán et al., 2020; Tayengwa et al., 2021; Villalba, Ates and MacAdam, 2021; Ravelo et al., 2022).

Despite the existence of several studies using alternative dietary sources to corn grain starch for dairy cows, studies with dairy does are still scarce. Some research evaluating non-fibrous carbohydrates in dairy goats are reported by Lunesu et al. (2021), who evaluated different performances of dairy does on dietary starch concentration during lactation. Regarding pectin, in diets composed by dry citrus pulp, Marcos et al. (2020) evaluated milk yield and composition, and observed improvements in milk, and both in milk fat and protein output. Sari, Naserian and Validazeh (2009) infused pectin and found no effect on DM and NDF digestibility. And Guzmán et al. (2020), used dehydrated orange pulp in the diet of dairy does to verify the effects on milk production and composition and found no differences on average milk production,.

Despite the knowledge of reduced pH in high-concentrate diets for dairy cows, we believe that goats tend to be more adaptable to NFC-rich diets. This can also be supported by Giger-Reverdin et al. (2020), who reported that rumen pH of goats is higher by about 0.4 units, compared to those recorded on cattle. Furthermore, goats appear to be more strategic, being able to decrease meal size and duration, select concentrates, and seek for fiber. They present longer chewing time per g of dry matter intake (DMI), about 10 times more if compared to cattle, and this difference can impact the recycling flow of bicarbonate/g of DMI, which might explain higher rumen pH values recorded for goats.

This study is the first to assess the effect of four different NFC sources in diets for lactating goats. We expect that the different sources of NFC (corn meal, pre-gelatinized corn, citrus pulp, and sucrose) used would not alter nutrient intake, digestibility, and milk yield and composition in lactating does.

2. Material and methods

2.1 Ethical aspects and location

The experiment was carried out at the experimental station of the Universidade Federal de Viçosa, located in the county of Viçosa, Minas Gerais, Brazil (20°46′19″S and 42°51′12″W; mean altitude 707 m), under an experimental protocol approved by the Ethics Commission on the Use of Farm Animals of Universidade Federal de Viçosa (CEUAP/UFV n° 063/2023 – Attachment A). According to the Köppen classification, the climate type is Cwa (tropical, high altitude), with rainy summers and dry winters. The annual mean temperature is 18.5 °C, ranging from 8.2 to 28.5 °C. The mean yearly pluviosity rate in this region is 1203 mm, with a relative humidity mean of 80%, according to Oliveira, Rodrigues and Fernandes, (2021).

2.2 Description of animals, diets and experimental design

We used 29 Saanen and Alpina dairy does, producing 2.8± 0.27 kg of milk/day, with 55.4± 1.8 kg of body mass, distributed in eight pens with four animals per pen. The pen was the experimental unit and the animals were observational units. The animals were randomly distributed to the pens, and pens formed a double-balanced 4 × 4 Latin square design with four treatments and four periods (Lucas, 1957). Each period consisted of 14 days for diet adaptation and 7 days for sample collection. The goats were placed in collective pens (5.30 x 5.90 m) with free access to water and mineral salt. Each pen was randomly assigned to one of four experimental diets. During the first period of the experiment, three goats ceased milk production, so they were removed from the experiment.

All diets were offered in the same physical form, that is, ground. The cornmeal was obtained from the feed factory of the UFV Agricultural Engineering Department. The pre-gelatinized corn, PGC was purchased from CIMILHO - Comércio Indústria de Milho Guimarães, Uberlândia- MG. The citrus pulp was initially obtained in pelletized form by the Santo Albano feed industry, Santos Dumont - MG. While sucrose in refined form was obtained by Companhia Agrícola Pontenovense Usina Jatiboca, Urucânia - MG. Diets based on corn silage (*Zea mays L.*), and concentrates were formulated to provide nutrients according to each carbohydrate source (Table 1).

Table 1. Proportion of ingredients of experimental diets in g/(kg^{-1})

Feeds	Diet 1	Diet 2	Diet 3	Diet 4
Corn meal	355,00	0,00	0,00	0,00
Soybean meal	554,00	554,00	562,00	512,00
Pré-gelatinized corn	0,00	355,00	0,00	0,00
Citrus pulp	0,00	0,00	365,00	0,00
Sucrose	0,00	0,00	0,00	449,00
Soy oil	43,00	43,00	49,00	0,00
NaCl	7,00	7,00	7,00	6,00
Bicarbonate	18,00	18,00	17,00	15,00
Limestone	22,00	22,00	0,00	18,00
Roughage:concentrate ratio	60:40	60:40	60:40	60:40

Table 2. Chemical composition of corn silage and diets

Variable[1]	Corn silage	Diet 1	Diet 2	Diet 3	Diet 4
DM	239.4±3.2	386.6±5.2	409.8±5.5	390.3±5.2	402.7±5.4
Ash	56.4±1.8	71.5±2.3	68.2±2.2	69.0±2.2	63.1±2.0
OM	943.65±2.0	928.50±2.0	931.80±2.0	931.00±2.0	936.90±2.0
CP	64.7±5.0	180.1±8.0	152.1±7.3	170.7±7.8	155.5±7.4
CF	18.5±0.31	18.9±0.32	17.8±0.31	18.5±0.31	19.4±0.32
aNDFom	504.0±11.7	309.8±10.5	298.8±10.4	335.8±10.9	270.6±10.0
ADL	74.0±5.5	65.1±7.5	70.6±4.2	80.4±9.8	75.2±11.6

[1] g/kg.

DM, Dry matter; OM, Organic matter; CP, Crude protein; CF, Crude fat; aNDFom, Neutral detergent fiber analyzed with a heat-stable amylase and reported without ash; ADL, Acid detergent lignin;

The criteria for allocating the animals to the different types of diets was milk production, with an average of 2 kg/day and the order of parturition, being primiparous (11) and multiparous (18), according to the data recorded in the UFV Capricornius software. The primiparous and multiparous goats were allocated to the pens, so that four pens received one primiparous and three multiparous goats, and the other four pens received two primiparous and two multiparous goats. The goats were introduced into the experiment 45 days after parturition and they were maintained up to the 129th day.

All experimental diets were formulated to meet the nutritional requirements of dairy goats and to be isoprotein (crude protein, CP, 150g/kg of dry matter, DM) and non-fibrous carbohydrates, NFC 390g/kg of DM, according to AFRC (1993), NRC (2001) and NRC (2007). To balance the diets, an electronic spreadsheet programmed in Microsoft Excel® was used, in which the Solver® tool was used (Jardim et al. 2013; 2015).

The goats were fed a total mixed ration, divided into four times a day, at 08:00, 11:00, 14:00 and 17:00 h, in amounts adjusted to allow for 10% orts, based on DM. On days 15 to 21 of each period, feed intake was determined by the difference between the quantities offered and orts. The animals were weighed in the morning, at the beginning and end of each experimental period, without feed and water restrictions, immediately after milking and before morning feeding on a mechanical scale (Chialvo, 502: S.M 300 kg, São Paulo, Brazil) to the nearest 0.005 kg.

2.3 Chemical analysis

Samples of diets and orts were collected on the 15th day of each period for seven consecutive days, homogenized, placed in identified plastic bags and stored in a freezer at -20 °C, and then were also used to determine the chemical analysis of DM, OM, MM, CP, CF, aNDFom, and ADL.

Subsequently, the samples were composed by treatment and the analyzes were carried out at the Animal Nutrition Laboratory - ANL, in Animal Science Department at UFV. Some analytical procedures were carried out in accordance with the National

Institute of Science and Technology - Animal Science (INCT- CA; Detmann et al., 2021). Samples were partially dried (INCT-CA G-001/2) and quantitatively ground in a Willey mill with a 1mm sieve, to then determine: dry matter values (DM; INCT-CA G-003/1), mineral matter (MM; INCT-CA M-001/2), crude protein (CP; INCT-CA N-001/2); and crude fat (CF; INCT-CA G-005/2).

At the Animal Science Laboratory of the Universidade Estadual do Norte Fluminense (UENF), ash analyzes of feces were carried out in porcelain crucibles (method 942.05; AOAC, 2019), equipped with porcelain lids to avoid spills during incineration. In addition to fibrous organic matter ($aNDF_{om}$), according to method AOAC 2002.04 (Mertens, 2002) and acid detergent lignin (ADL), according to method AOAC 973.18 (Möller, 2009).

The aNDFom and ADL methods were strictly followed, such as the use of a Berzelius beaker without spout (600 mL, high form), for use in digestion units with condensers (model MA450/6; Marconi Equipamentos para Laboratórios LTDA, Piracicaba, SP, Brazil), addition of sodium sulfite, two additions of standardized amylase solution, and filtration in Gooch crucibles (coarse porosity and filtration rate tested) to determine fiber in feeds and orts, and use of filter aid for faecal samples (Mertens, 2002; Möller, 2009). The standardized amylase solution was prepared from the commercial concentrated solution (α-amylase Liquozyme® Supra 2.2x de *Baccilus licheniformis*, Tecnoglobo, Curitiba, PR, Brazil, licensed by Novozymes A/S, Denmark).

2.4 Digestibility

To determine digestibility, two goats were randomly selected per pen for sampling. Fecal production per animal was determined on the 15th day and for the next six consecutive days, and were immediately taken (zero hours), and at 4 h-intervals until 48 h, then in a sequence of 8 h-intervals until 96 h, at 12 h-intervals until 144 h, and finally at 192 h according to (Vieira et al. 2020). Feces were collected after stimulation directly from the rectum, and were homogenized, placed in plastic bags and stored at -15 °C, and subsequently composited faecal samples were obtained. Afterwards, fecal samples were partially dried (INCT- CA G-001/2) and quantitatively ground in a Willey-type mill with a 1mm sieve, to then determine DM values (DM; INCT-CA G-003/1) (Detmann et al., 2021).

Fecal samples were weighed (to the nearest 0.1 mg) and subjected to acid detergent, and the residue recovered was treated with a 72% sulfuric acid solution. The final residue recovered represented the ADL, corrected for ash, which was used as an internal marker. The choice of lignin was due to its cost/benefit ratio and also because it is less invasive, reinforcing that to obtain the best results, the use of filter aid in the crucibles is essential. The correction for ash was carried out in a muffle furnace with an electronically programmed temperature (model MA385/3; Marconi Laboratories equipment LTDA, Piracicaba, SP, Brazil).

ADL methods were strictly followed, using Gooch crucibles (coarse porosity and filtration rate tested) to determine lignin in faeces. In addition to the use of a filter aid (glass microfiber prefilter, GF/D Whatman™, 42.5 mm circle) for fecal aNDFom determination (Mertens, 2002). These data were used to estimate total fecal production and DM, OM, and aNDFom of digestibility coefficients. The apparent digestibility of each nutrient was calculated as the intake of a nutrient (kg/d) minus the fecal excretion of that nutrient (kg/d) divided by the intake.

2.5 Production and composition of goat milk

Individual milk production was monitored for seven days of each experimental period, with daily production corresponding to the sum of two milkings per animal. The animals were manually milked twice a day (06:00 a.m and 14:30 p.m). No medication was administered to induce milking. Before milking, the first streams of milk were discarded, and the teats were disinfected with a cleaning solution based on glycerin and lactic acid and dried with paper towels. Milk production (to the nearest 0.1 g) was determined by weighing on a digital scale (Toledo Brazil®, model 2098/61, 60kg, São Paulo, Brazil).

Samples for milk composition from the morning and afternoon milkings were taken on the 1st, 3rd and 7th day per animal per experimental period. They were subsequently homogenized to form a composite sample and placed in sterile plastic containers containing brononata® (Laborclin Laboratory products Ltda, Pinhais, PR, Brazil) preservative. They were identified and stored in a thermal box with recyclable ice until delivery to the laboratory. Sample collection and sending procedures were adopted in accordance with the recommendations of the instruction manual of the Clínica do Leite (ESALQ/USP), where the following composition analyzes were carried out: fat, total protein, lactose, total solids, total solids non-fat, these analyzes being carried out by mid-infrared absorption spectrometry, and results expressed in g/100 g of raw milk (%) (ISO 9622:2013/IDF 141:2013) (International Organization for Standardization, 2013). To determine the somatic cell count (SCC), the flow cytometry method was used (ISO 13366-2:2006/ IDF 148-2:2006), the results being expressed in somatic cells/mL (International

Organization for Standardization, 2006). The correction of milk to 4% fat was carried out according to NASEM (2021).

2.6 Ingestive behavior

Individual observations of the animals were carried out on four different days at the beginning and end of the last experimental period at five-minute intervals to evaluate ingestive behavior according to the method of Martin and Bateson, (1993) and Bateson and Martin, (2021). The behavioral activities recorded for each animal were: ruminating, eating, drinking, and idleness. Observations were assumed to last the entire interval and were recorded by four trained people using a digital stopwatch, positioned to minimize interference with behavior. For nocturnal observation of the animals, the environment was kept artificially lit. Animals were marked with paint to facilitate identification and allow observers to maintain their distance to ensure minimal interference with the animal behavior.

2.7 Quantitative methods

The dry matter intake $\bar{F}_i^S$, represents the average daily intake rate of DM as g/d (no scaled), and when scaled to body weight, (BW), it was represented by $\bar{F}_i^S$ expressed as $g/(kg \cdot d)$, and average daily intake rates of organic matter, (OM), $\bar{F}_{OMi}^S$, ash, $\bar{F}_{ashi}^S$, crude protein, (CP), $\bar{F}_{CPi}^S$, crude fat, (CF), $\bar{F}_{CFi}^S$, fibrous organic matter, (aNDFom), $\bar{F}_{NDFi,}^S$ and acid detergent lignin, (ADL), $\bar{F}_{ADLi,}^S$ were expressed as $g/(kg^{0.75} \cdot d)$. The Gamma distribution function of the family of exponential distributions expands the modeling capabilities of linear models and was assumed to interpret data in this study. Therefore, scaled intake rates were predicted by assuming that $y_{hlmn}|p,a \sim \Gamma(\mu_{hlmn}, \Phi)$, with mean μ_{hlmn}, shape parameter Φ, variance $\Phi\mu_{hlmn}^2$, link function $\log \mu_{hlmn}$, and a linear predictor as follows (Stroup, 2013):

$$\xi_{hlmn} = \xi + \alpha_h + p_l + \tau_m + \alpha\tau_{hm} + a_{n(l)}. \quad \text{Eq.(1)}$$

In this model framework, Greek letters denote fixed effects and Latin letters denote random effects. y_{hlmn}, is the recorded intake when the n-th animal ($a_{n(l)}$) within the l-th pen consumed the h-th treatment (α_h) during the m-th period. The term $\alpha\tau_{hm}$ accounts for a possible interacting treatment x period effect. Random effects are assumed normally, identically, and independently distributed ($\mathcal{N}$), as $a_{n(l)} \sim \mathcal{N}(0, \sigma_a^2)$, and $p_l \sim \mathcal{N}(0, \sigma_p^2)$.

The BW was analyzed with the same statistical framework as intake rates, because of possible heterogeneous variances of errors for the four treatments. However, the concentration of nutrients in the intake, C_i, in organic matter, OM, C_{OM}, in ash C_{ash}, in crude protein, CP, C_{CP} , in crude fat, CF, C_{CF} , in fiber organic matter, NDF C_{NDFi}, acid detergent lignin C_{ADL}, (g/kg, DM basis), and concentration of milk components were analyzed by assuming a Beta distribution, the same linear predictor as Eq. (1), and a logit link function (Silva et al., 2018). The SCC in milk was analyzed assuming a negative binomial the same linear predictor as Eq. (1), and a log link function. These models were fitted by residual pseudo-maximum likelihood (RSPL) using the GLIMMIX procedure of SAS (SAS® Studio release 3.81, SAS Institute Inc., Cary, NC, USA) with Tukey adjustment for the type I error rate. Statistical differences were declared significant at $P < 0.05$.

3. Results

All variables nutrient intake and digestibility had an interaction between diet and period. About, the effects of milk yield and composition, it is also observed an effect of diet x period interaction for milk yield (MY), previous milk yield (MYp), lactose and total solids non-fat (TSNF). However, most interactions when broken down do not show a significant effect, only acid detergent lignin intake, ADLI. Some variables had only an effect of diet, such as: fat; protein; total solids; TS; fat corrected for milk; FCM and net energy of lactation; NEL. And only fat, FCM and NEL were influenced by the period.

No difference was observed in somatic cell counts between diets and periods evaluated. According to the results of P–random values of the covariate of previous milk yield and ingestive behavior, we did not observe significant effects, as presented as supplementary material (see Appendix A). Fixed p values for ingestive behavior also are shown in Table 3.

Table 3. P–values of fixed effect and interactions for body weight, nutrients intake from diets, digestibility, and milk yield and composition

Variable	Fixed effect		Interaction
	Diet	Period	Diet x Period
Body weight and nutrient intake			
BW[2]	0.948	0.004	0.485
$\bar{F}_i^S$ [1]	<0.001	<0.001	0.002
$\bar{F}_i^S$ [2]	<0.001	<0.001	0.028
$\bar{F}_{OMi}^S$ [2]	0.002	<0.001	0.032
$\bar{F}_{CPi}^S$ [2]	<0.001	<0.001	0.019
$\bar{F}_{CFi}^S$ [2]	<0.001	<0.001	0.017
$\bar{F}_{NDFi}^S$ [1]	<0.001	<0.001	<0.001
$\bar{F}_{NDFi}^S$ [2]	<0.001	<0.001	<0.001
$\bar{F}_{ADLi}^S$ [2]	<0.001	<0.001	<0.001
Digestibility			
DMD[3]	<0.001	<0.001	<0.001
OMD[3]	<0.001	<0.001	<0.001
NDFD[3]	<0.001	<0.001	<0.001
Milk yield and composition			

MY[4]	0.001	0.791	0.037
MYp[4]	0.001	0.793	0.040
FCM 4%[4]	<0.001	0.019	0.209
Fat[5]	<0.001	0.001	0.551
Protein[5]	0.005	0.336	0.075
Lactose[5]	<0.001	0.239	0.033
TS[5]	<0.001	0.112	0.087
TSNF[5]	0.003	0.388	0.041
SCC[7]	0.308	0.290	0.936
NE_L[6]	<0.001	0.035	0.164

[1] $g/(d \cdot kg^{-1})$; [2] $g/\left(d \cdot kg^{3/4}\right)$; [3] Dimensionless; [4] (kg/d); [5] (g/d); [6] (MJ/d).

BW, Body weight; $\bar{F}_i^S$, averaged daily intake rate of DM, $\bar{F}_{OMi}^S$ averaged daily intake rate of organic matter, OM, $\bar{F}_{ashi}^S$, averaged daily intake rate of ash, $\bar{F}_{CPi}^S$, averaged daily intake rate of crude protein, CP, $\bar{F}_{CFi}^S$ averaged daily intake rate of crude fat, CF, $\bar{F}_{NDFi,}^S$ averaged daily intake rate of fibrous organic matter, $\bar{F}_{ADLi,}^S$ averaged daily intake rate of acid detergent lignin, ADL, expressed as $g/(kg^{0.75} \cdot d)$ DMD, Dry matter digestibility; OMD, Organic matter digestibility; NDFD, Neutral detergent fiber digestibility; MY, Milk yield; MY_P, Milk yield previous; FCM, Fat-corrected milk; TS, Total solids; TSNF, Total solids non-fat; NE_L, Net energy of lactation.

3.1 Body weight and nutrients intake

The body weight of the goats was influenced throughout the experimental period (Table 4), being smaller in the 1st and bigger in the 4th period.

Table 4. Effects of body weight $g/\left(d \cdot kg^{3/4}\right)$ on period

Period	Body weight
1	19.6±0.39b
2	20.2±0.40ab
3	20.7±0.41ab
4	21.3±0.42a

Values are presented as means.

3.2 Digestibility

When we observe the ADLI, it can be emphasized that the diet with corn meal was greater in periods 3 and 4. However, the diets with sucrose was lower in most periods evaluated (Table 5).

Table 5. Result of breakdown of factors (Diet x Period) of nutrients intake and digestibility coefficient of goats fed non-fibrous carbohydrate diets

$\overline{F}^{S}_{ADLi}$ $g/(d \cdot kg^{3/4})$				
Diet	Period			
	1	2	3	4
Corn meal	0.50±0.04Bb	0.77±0.06ABab	0.99±0.07A	1.19±0.09Aab
PGC	0.96±0.07a	0.72±0.05b	0.96±0.07	1.00±0.07ab
Citrus pulp	0.78±0.06Bab	1.31±0.10ABa	1.23±0.09AB	1.64±0.12Aa
Sucrose	0.56±0.04Bab	1.34±0.10Aa	0.73±0.05B	0.83±0.06ABb
Dry matter digestibility coefficient $g/(d \cdot kg^{3/4})$				
Corn meal	849.5±2.3Aa	768.3±3.0Ba	711.0±3.4Cc	695.9±3.5Cc
PGC	742.9±3.2Ad	768.9±3.0Ba	739.2±3.2Bb	748.1±3.2Bb
Citrus pulp	818.8±2.6Ac	721.3±3.3Bb	731.7±3.3Bb	686.4±3.5Cc
Sucrose	833.5±2.5Ab	634.9±3.8Cc	801.4±2.8Ba	790.2±2.9Ca
Organic matter digestibility coefficient $g/(d \cdot kg^{3/4})$				
Corn meal	862.2±2.4Aa	787.9±3.1Ba	727.7±3.6Cc	718.7±3.7Cc
PGC	760.3±3.4Bd	794.5±3.1Aa	759.7±3.4Bb	761.5±3.4Bb
Citrus pulp	828.5±2.7Ac	750.8±3.5Bb	748.4±3.5Bb	704.6±3.8Cc
Sucrose	845.9±2.5Ab	669.1±4.0Cc	816.4±2.9Ba	808.2±2.9Ba
Neutral detergent fiber digestibility coefficient $g/(d \cdot kg^{3/4})$				
Corn meal	674.5±7.5Aa	602.4±7.9Ba	568.1±7.9Bb	436.5±7.9C
PGC	504.9±8.0Bc	560.2±7.9Ab	488.8±8.0Bc	447.1±8.0C
Citrus pulp	695.1±7.3Aa	551.2±7.9Bb	577.6±7.9Bb	429.9±7.9C
Sucrose	630.6±7.7Ab	331.8±7.5Cc	618.8±7.8Aa	457.4±8.0B

ADLI, Acid detergent lignin intake; PGC, pregelatinized corn. Values are presented as means ± SEM. Means followed by different letters, lowercase in the column and uppercase in the row, differ by Tukey's test by 5%.

When we observe the dry matter digestibility coefficient, DMDC, organic matter digestibility coefficient, OMDC, the corn meal diet obtained the highest digestibility in the periods one and two, and the lowest in periods three and four. While the sucrose obtained the lowest of DMDC, OMDC and NDFD in the period two. About NDFD, we can see that the diet with corn meal and citrus pulp obtained the highest DMDC, OMDC and NDFD in period one, as shown (Table 5).

3.3 Milk yield and composition

When we only observe diet effect, the citrus pulp presented the highest values of milk composition, including fat, protein, TS, FCM, and NE_L. However, we found that sucrose diets interfered with the low values of milk composition for all the variables mentioned above (Table 6).

Table 6. Effects of diets and periods on milk composition of goats fed non-fibrous carbohydrate diets

Diets	[2]Fat	[2]Protein	[2]TS	[1]FCM	[3]NE_L
Corn meal	88.4±7.8b	86.7±6.6ab	328.0±25.9b	2.5±0.21b	7.6±0.62b
PGC	87.2±7.7b	88.9±6.8a	331.5±26.1b	2.5±0.21b	7.7±0.62b
Citrus pulp	109.2±9.7a	91.7±7.0a	363.2±28.7a	2.9±0.24a	8.7±0.70a
Sucrose	70.6±6.2c	80.6±6.1b	292.9±23.1c	2.2±0.18c	6.6±0.53c

Period	[2]Fat	[1]FCM	[3]NE_L
1	92.5±8.2a	2.6±0.21a	7.9±0.64a
2	92.4±8.2a	2.6±0.21ab	7.7±0.63ab
3	86.4±7.6ab	2.5±0.20ab	7.5±0.60ab
4	80.5±7.1b	2.4±0.20b	7.3±0.59b

[1] kg/d; [2] g/d; [3]MJ/d

TS, Total solids; FCM, Fat-corrected milk; NE_L, Net energy of lactation; PGC, pregelatinized corn. Presented values are presented as means ± SEM. Means followed by different letters, differ by Tukey's test by 5%.

We observed that the periods had little influence on milk composition. Only fat, FCM and NE_L varied, in which period one presented the highest values, while period four showed the lowest results (Table 6).

3.4 Ingestive behavior

There was no effect of diet for rumination and drinking behaviors. We observed that there was a greatest intake effect, when goats were fed the corn meal diet, and shorter for the PGC diet. The idle was influenced by diet, being higher for goats fed with PGC, and lower when fed corn meal (Table 7).

Table 7. Effects of diets on ingestive behavior (h/day) of goats fed non-fibrous carbohydrate diets

Diet	Rumination	Intake	Drinking	Idle
Corn meal	6.3±0.58	4.6±0.29a	0.33±0.07	12.2±0.39b
PGC	5.3±0.48	3.5±0.22b	0.16±0.04	14.5±0.45a
Citrus pulp	5.9±0.55	4.3±0.30ab	0.24±0.05	12.9±0.43ab
Sucrose	6.6±0.64	3.6±0.27ab	0.16±0.04	12.8±0.45ab
P - value	0.493	0.017	0.253	0.005

PGC, pregelatinized corn. Values are presented as means ± SEM. Means followed by different letters, differ by Tukey's test by 5%.

4. Discussion

4.1 Body weight and Nutrients intake

Despite the observed weight increase, we emphasize that we should not consider this variable, as short evaluation periods, present in Latin square design tend to difficult weight gain variation, not correcting rumen filling errors and affecting weight gain accuracy (Eaton, Gosslee and Lucas, 1959).

In our study, the $\bar{F}_i^S$ was not influenced by different types of non-fibrous carbohydrates (Table 3). Although we did not observe an effect of $\bar{F}_i^S$ between the diets, we believe that the higher $\bar{F}_{ADLi}^S$ in the corn meal diet may be associated with the higher $\bar{F}_i^S$ in the corn meal diet. Unlike the others, the goats needed to ingest larger quantities to meet their energy needs, thus reaching satiety, as reported by Carvalho et al. (2006).

4.2 Digestibility

For Oliveira et al. (2014), increased feed digestibility leads to greater availability of nutrients for milk production. Therefore, diet digestibility can be reduced as the total DMI is increased. Although ruminant saliva does not contain amylase, we believe that the higher DMDC for corn meal is associated with a greater quantity of amylolytic bacteria, which fixed and colonized the grain particles, similar to the observations made by Nozière et al. (2010). Furthermore, high amounts of starch being digested in the rumen reduces the flow of starch to the duodenum (kg/d) and optimizes starch hydrolysis in the small intestine (Ferraretto, Crump, Shaver, 2013).

Oba and Main (2023) also reported that the site of starch digestion affects the type of absorbed fuel. When a slowly fermentable starch source, such as dry ground corn, is used to feed dairy cows, ruminal starch digestibility is decreased substantially, but total tract starch digestibility is not affected, as much due to partial compensatory digestion in the intestines, indicating that substantial amounts of starch are digested in the intestines. If starch is digested enzymatically in the small intestine, it is absorbed as glucose, which increases glucose or lactate flux to the liver. Type of absorbed fuels (propionate, glucose, or lactate) affects the extent of hepatic oxidation and feed intake. As such, temporal pattern of fuel supply from carbohydrate digestion needs to be integrated in predictions of DMI and fuel metabolism.

The better NDF digestibility in citrus pulp diet for dairy goats can be attributed to the types of carbohydrates present in citrus pulp. In our study, the chemical composition of the citrus pulp diet showed the highest fiber values (Table 2). However, citrus byproducts contain a variety of energetic substrates for ruminal microorganisms, including soluble carbohydrates readily digestible, like pectins (Bampidis and Robinson, 2006; Sharif et al., 2018).

In our study, we observed lower DMDC and OMDC for PGC and sucrose diets. It was similar to Campos et al. (2020), who evaluated different sources and doses of sugars in ruminant digestibility and observed marked reductions in fibrous portions digestibility, being more pronounced in doses of sugars such as sucrose, xylose, fructose and glucose.

However, we noticed a depression in fiber digestibility when sucrose was used. We believe that the lower neutral detergent fiber digestibility coefficient, NDFDC of sucrose and PGC may be associated with the low fiber values found in the composition of these diets (Table 2). This observation is reported by Keim et al. (2022), when evaluating the replacement of ground corn with sugar beet in the diet of pasture-fed dairy cows, also emphasizing that sugar has often been associated with a reduction in fiber digestion.

Hall and Zanton (2022) evaluated the digestibility of NDF and observed that there was no difference between 0 and 3% molasses, but there was an increase when using 6% and a reduction with 9% inclusion. In the same study, the digestibilities of DM, OM and NDF increased linearly with increasing inclusion of dry molasses at 0, 4, 8 and 12% of the diets DM. However, the mechanisms of this oscillating behavior are not yet well described in the literature.

4.3 Milk yield and composition

We did not observe any diet effect on milk yield. Such data corroborate with López et al. (2014), when evaluating the use of citrus pulp or soybean hulls to replace corn grain, on milk performance in lactating Murciano-Granadina goats, in which no significant difference was observed in milk production (1.72 kg/d).

We did not observe a lactose effect among the diets, and this is in accordance with the milk yield data observed in our study, which also did not differ, since lactose content directly influences milk volume. Antanaitis et al. (2024), observing lactose concentration of in cow's milk, emphasized that lactose plays a crucial role as an osmotic agent in milk, significantly influencing the transfer of water from the bloodstream to the milk. Furthermore, lactose synthesis occurs within the udder, where blood glucose is absorbed by the basement membrane of mammary epithelial cells and then converted into lactose. Therefore, a reduced level of lactose leads to a decrease in milk volume production.

Citrus pulp, in general, had a great influence on milk components in our study. We can infer that this is due to the increase in ruminal acetate concentration, since acetate is an important precursor for the *de novo* synthesis of milk fat (Arthington, Kunkle and Martin, 2002, Münnich et al. 2017). Our results are similar to those of Volanis et al. (2006), who evaluated the use of a mixture of ensiled citrus pulp to lactating dairy sheep and observed that milk fat content was 17% higher than that of sheep fed a conventional diet, thus highlighting that citrus by-products improve the use of fibrous fractions of the diet, by causing fiber degradation, possibly due to their positive effects on rumen microflora of the. Better fiber degradation provides the production of acetic acid which, in turn, promotes milk fat synthesis.

Pascual and Carmona (1980) report that citrus pulp protein is one of the constituents that varies most in citrus production, since it can be influenced by the seed fraction, as high levels of LDA tend to reduce the protein. In addition, the source of the fruit and the type of processing (Salvador et al., 2014) can also influence. We consider that the higher value of milk protein may be associated with the type of citrus pulp processing, since the product was purchased in pelletized format. Lima et al. (2023b), also report which the ruminal microbial protein synthesis provides at least half of the amino acids for milk protein synthesis in ruminants.

The FCM production can be optimized when levels of sucrose are added to starch-rich diets (Klevenhusen and Zebeli, 2021). This is different from our work, in which sucrose influenced the lower FCM. The decline of some milk constituents in goats fed sucrose diet may be related to DMI because, although it was not significant, it tended to be lower when compared to the other diets. Furthermore, for Hall and Zanto (2022), sucrose can ferment into lactate, and lactate can be converted by ruminal microbes into propionate and acetate, but preferably into propionate in diets containing high level of concentrate. Another effect of sugars, related to the production of milk fat, is rumen biohydrogenation. Microbes that utilize sugar (glucose, maltose, cellobiose) in rumen are capable of fatty acid (FA) biohydrogenation, which can reduce the amount of bioactive unsaturated FA reaching the intestine, which could depress milk fat production. However, our diets did not have high fat content, so it is not clear whether this could be the primary factor in the fat response.

The lower fat and FCM contents in period 4 may be associated with several factors, including the advance of the animals in lactation, since the experimental evaluation with the diets began after the lactation peak. For Oliveira, Rodrigues and Fernandes (2021), several factors are related to the animal (breed, genetic selection, age, number of pregnancies, lactation stage) and environmental factors (health, heat stress, nutrition and photoperiod) that affect milk composition. However, the reduction in milk fat content is related to at least two phenomena, the dilution effect due to the increase in milk volume at lactation peak and the reduction in fat mobilization as the weeks of lactation advance. As a result, there is a reduction in non-esterified fatty acids (NEFA) in the mammary gland, which can cause a decrease in milk fat synthesis.

4.4 Ingestive behavior

Ingestive behavior allows us to understand the variations that may occur during feed intake (Lima et al., 2023a). In this study, we observed that rumination time did not change between diets. Therefore, it is suggested that there was an influence of the diet due to the same proportion of non-fibrous carbohydrates, although coming from different sources. In addition, we also attribute the physical form, ground, that the diets were offered.

The greater intake of corn meal in the diet may be associated with the animals need to increase intake to meet the energy requirement, since other diets possibly make energy available faster (Carvalho et al., 2006). We observe that the goats fed with PGC had longer idle time, due to low and rapid intake. This was also reported by Sermento et al. (2011), who reported that goats fed diets containing 60% concentrate tend to modify their feeding behavior, spending less time eating and ruminating, and more time resting, after distribution of the meal.

Findeisen et al. (2021), when evaluating the increase in feed intake in domestic goats, report that increased intake tends to lead to an increase in intestinal filling and a reduction in digesta retention times. Furthermore, animals adjust their chewing intensity to the food they eat.

5. Conclusion

Diets with non-fibrous carbohydrates resulted in different effects. We suggest that citrus pulp could be used in feeding goats without compromising the nutrients intake and digestibility and milk yield and composition. However, a sugar diet compromises the composition of milk.

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7. GENERAL CONCLUSIONS

The use of non-fibrous carbohydrates has been widely studied in dairy cows, but few data are found in the literature on dairy goat diet. We believe that goats are more resistant to diets high in non-fibrous carbohydrates, but the mechanisms are still underreported and controversial, with varying performance data.

APPENDIX A

Table 1. P–values of previous milk yield and ingestive behavior initial variables.

Previous milk yield and composition	
[1]MYp	0.402
[1]FCM 4%	0.410
[2]Fat	0.403
[2]Protein	0.413
[2]Lactose	0.377
[2]TS	0.393
[2]TSNF	0.387
[3]NE_L	0.403
Ingestive behavior	
[4]Rumination	0.058
[4]Intake	0.513
[4]Drinking	0.399
[4]Idle	0.053

[1](kg/d); [2] (g/d); [3] (MJ/d); [4] (h/day).

MY_P, Previous milk yield; FCM, Fat-corrected milk; TS, Total solids; TSNF, Total solids non-fat; NE_L, Net energy of lactation.

ATTACHMENT A

UNIVERSIDADE FEDERAL DE VIÇOSA
COMISSÃO DE ÉTICA NO USO DE ANIMAIS DE PRODUÇÃO
CEUAP/UFV

Campus Universitário – Viçosa, MG – 36570-900 – Telefone:(31) 3899.3275 – e-mail: ceuap@ufv.br – site: www.ceuap.ufv.br

Viçosa, 06 de Outubro de 2023

CERTIFICADO

Certificamos que o projeto intitulado **"Avaliação de diferentes tipos de carboidratos não fibrosos na dieta de cabras leiteiras"**, protocolo **nº 063/2023**, sob a responsabilidade de **Marcelo Teixeira Rodrigues** - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo chordata, subfilo vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 8 de outubro de 2008, do decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo conselho nacional de controle da experimentação animal (concea), e foi apreciado pela comissão de ética no uso de animais de produção da universidade federal de viçosa (ceuap-ufv) em reunião de **12 de Julho de 2023.**
Finalidade: **(x)Pesquisa ( )Ensino**
Vigência do Projeto: de **01 de agosto de 2023** a **01 de Junho de 2024**
Espécie/linhagem: **Cabra (Capra hircus)** Nº de animais: **32**
Peso: **53Kg** Idade: **2 anos** Sexo: **Fêmea** Origem: **Setor de Caprinocultura - DZO /UFV - CNPJ/CPF: 25.944.455/0001-96**
Data de Aprovação: **06 de Outubro de 2023**

CERTIFICATE

We certify that the project entitled **"Evaluation of different types of non-fibrous carbohydrates in the diet of dairy goats",** protocol **nº 063/2023**, under the responsibility of **Marcelo Teixeira Rodrigues** - which involves the production, maintenance and/or use of animals belonging to the phylum chordata, subphylum vertebrata (except man), for scientific research purposes (or education) - is in accordance with the law nº. 11.794, of October 8, 2008, Decree nº. 6899 of July 15, 2009, and the rules issued by the Brazilian National Council for Animal Experimentation Control (CONCEA), and was approved by the Ethics Commission on the use of farm animals of Universidade Federal de Viçosa (CEUAP-UFV) in its meeting on **Jul. 12Th of 2023**.
Finality: **(x)Research ( )Education**
Duration of the Project: from **Aug. 08Th of 2023** to **Jun. 01Th od 2024**.
Species / strain: **Goat (Capra hircus)** Nº of animals: **32**
Weight: **53Kg** Age: **2 years** Sex: **Female** Source: **Setor de Caprinocultura - DZO /UFV - CNPJ/CPF: 25.944.455/0001-96**
Approval date: **Oct. 06Th of 2023**

Luciana Navajas Rennó
Luciana Navajas Rennó
Coordenadora da CEUAP/UFV