Goat Milk Whey Improves Nutritional Status, Fecal Microbial Composition and Intestinal Morphology in Female Rats Fed a Westernized Diet and Their Offspring

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Abstract This study evaluated whether supplementation with goat milk whey (GMW) affects the nutritional status, intestinal histology and intestinal microbial composition of female rats fed a westernized diet from gestation to lactation, as well as whether changes can be replicated in the offspring at the end of weaning and at 45 days of life. Pregnant female rats were randomized into four groups: control group (CSAL), control group supplemented with GMW (CGOAT), westernized group (WSAL) or westernized group supplemented with GMW (WGOAT). After weaning, half of offspring were euthanized and the rest of the offspring were maintained under the same treatment applied to dams, up to 45 days of life. Body weight, food intake, intestinal histology and counting of fecal microbial groups were determined in both female rats and offspring. The offspring supplemented with GMW showed decreased body weight at weaning. After weaning, groups supplemented with GMW showed reduced body weight and visceral fat, increased fecal lactobacilli counts in rats and offspring and attenuation of damages induced by the westernized diet on intestinal epithelial cells. GMW supplementation caused a positive effect on fecal microbial composition, intestinal morphology and induced reduction in weight gain and visceral fat in female rats and offspring fed westernized diet. These effects appear to be dependent on the animal's age and period time of GMW supplementation.

Keywords: *westernized diet, goat milk whey, nutritional status, fecal microbial composition, small intestine*

1. Introduction

A westernized diet is characterized by excess lipids, simple carbohydrates and sodium, in addition to reduced dietary fiber and high consuming of processed foods [1]. Experimentally, westernized diet consumption is associated with metabolic disorders such as non-alcoholic fatty liver disease [2]. Westernized diet intake during pregnancy and the lactation period predisposes offspring to increase body fat and metabolic syndrome disease [3]. Fat-rich diet, mainly saturated fat, promoted changes in body fat and intestinal morphology, leading to intestinal villi wear [4] and change in intestinal microbiota [5]. In addition, were observed associate with neuroinflammation and cognitive dysfunction [6] and brain health and correlated diseases [7]. However, it has been advocated that the negative impacts of westernized diets consumption on intestinal health and microbial composition may be minimized by consuming components such as amino acids, prebiotics, probiotics, and postbiotics [8], fiber and oligosaccharides [9] increasing the population of beneficial microorganisms (e.g, bifidobacteria and lactobacilli) in the gut environment.

Supplementation with cow milk and whey showed positive effects on the counts of bifidobacteria and lactobacilli in rat feces [10,11], as well as oligosaccharides which improved inflammation and dysbiosis in obese mice fed a westernized diet [9]. In the breast milk, the oligosaccharides were capable of modulate intestinal microbial composition by selectively stimulating the growth of beneficial bacteria in the gut environment

[12,13]. Goat milk seems similarly affect the intestinal microbial composition despite presenting lower oligosaccharides amounts than breast milk [14]. And more, dietary whey proteins also showed resistance the installation of dysbiosis promoved by high-fat diet [10]. However, there is a lack of studies on the effects of supplementation with goat milk whey. Studies investigating the potential benefits of goat milk whey are important because it is a highly produced by-product from the goat dairy industry that needs an adequate and value-added destination [15].

Considering that pregnancy and lactation are critical and highly vulnerable periods to environmental influences, which may affect short- and long-term development [16] and health, this study tested the hypothesis that supplementation of goat's whey will may minimize the effects caused by the Westernized diet on the fecal microbial composition of mothers and pups and adverse metabolic outcomes. Thus, the goal of this study was to evaluate whether supplementation with goat milk whey improves the nutritional status, fecal microbial composition and intestinal morphology of female rats and offspring fed a westernized diet from gestation and lactation until the end of the highest offspring growth phase.

2. Methods

The experimental protocol was approved by the Ethics Committee of the Biological Sciences Center (protocol no. nº 23076.044893/2014-21), Federal University of Pernambuco, Recife, PE, Brazil and followed the Guidelines for the Care and Use of Laboratory Animals [17].

2.1. Goat Milk Whey

Goat milk whey (GMW) used in the experiment was obtained from the Goat Breeding Sector of the Federal University of Paraiba (Bananeiras, Brazil). Goat milk samples were collected on five consecutive days by manual milking in an appropriate room. The goat milk was processed to obtain a medium-to-high moisture fresh cheese, and the generated sweet-type GMW was obtained as a processing by-product. Approximately 7L of GMW were first defatted and filtered through triple-layer cheesecloth to remove gross contaminants. GMW was frozen at -20°C and freeze-dried under vacuum pressure (150 µHg, -47°C for 24 h) using a bench lyophilizer (L101 Liotop®, São Carlos, Brazil) [15]. The freeze-dried GMW was stored in glass packaging in freezing temperature and analyzed in triplicate regarding the moisture, ash, proteins, lipids and lactose contents [18]. The gross composition of tested GMW is shown in Table 1. Freeze-dried GMW was submitted to analysis for enumerating total and fecal coliforms, coagulase-positive Staphylococcus, *Lactobacillus* spp., *Bacteroides* spp., *Bifidobacterium* spp. and Enterobacteria, as well as for evaluating the absence/presence of Salmonella, using standard procedures [19]. Coagulase-positive Staphylococcus, *Lactobacillus* spp., *Bacteroides* spp., *Bifidobacterium* spp. and Enterobacteria counts were < 1 log cfu/mL, and *Salmonella* spp. was absent in GMW.

Table 1. Physicochemical characteristics of the freeze-dried goat milk and milk whey

Parameters	Goat milk Mean ± SD	Goat milk whey Mean ± SD
Protein (%)	3.15± 0.05 %	15.78 ± 0.52
Carbohydrates (%)		
Lactose (%)	4.69± 0.08 %	9.95 ± 0.37
Others	n.d.	n.d
Lipid (%)	2.68± 0.02 %	11.40 ± 0.74
Moisture (%)	89.08± 0.12 %	4.14 ± 0.02
Ash (%)	0.70 ± 0.02 %	6.88 ± 0.70
Energetic value (Kcal)	55.48	205.52

From: Analysis by Laboratory of Experimentation and Food Analysis (Federal University of Pernambuco, Recife, Brazil)
*n.d = not determined.

2.2. Animals and Treatments

The animals received water and diet ad libitum throughout the experimental period and were kept in standard lighting conditions (light/dark cycle of 12/12 h, starting light cycle at 06:00 am), temperature of 22 ± 1°C and environmental humidity of 65%. Experimental procedures followed standard procedures established by the National Council for Animal Experimentation Control (CONCEA - Brazil). The experiment was realized in dams at end lactation, in pups at 23 days old (stage 1 or first phase of experiment) and in pups at 45 days old (stage 2 or second phase of experiment).

Table 2. Chemical and energy composition* of control and westernized diet consumed by female rats and offspring during the experiment

Chemical composition	Control Diet	Westernized diet
Carbohydrates (g/100 g) of which:	59.9	49.6
Simple carbohydrate	10.0	23.0
Complex carbohydrate	44.0	22.0
Fibers	5.0	4.6
Lipids (g/100g) of which:	7.2	19.0
Saturated (%)	26.0	67.6
Monounsaturated (%)	12.4	16.5
Polyunsaturated (%)	61.6	15.9
Proteins (g/100 g)	17.0	22.0
Moisture and volatile substances	9.5	4.2
Ash (g/100g)	6.0	5.2
Total Energy (kcal/g)	3.6	4.1
% kcal TE		
Protein	19.0	21.0
Carbohydrate	63.0	42.0
Lipid	18.0	37.0

*From: Analysis by Laboratory of Experimentation and Food Analysis (Federal University of Pernambuco, Recife, Brazil).

Eight male and 24 females (220-240 g) Wistar rats (Rattus norvegicus, albinus variety) were used to perform mating in the ratio of 1 male:3 females. The day on which spermatozoa were present in a vaginal smear was designated as the day of conception, day 0 of pregnancy. The evolution of gestation was realized by increase of body weight of females, who randomly was subdivided into four groups during the gestation period (21-23 days)

in accordance with the offered diet (Table 2) [20] and supplementation or not with GMW: Then, four groups (n=5-6/each) were formed as follow: CSAL (animals fed AIN-93G control diet and gavage with saline); CGOAT (animals fed AIN-93G control diet and supplementation with GMW); WSAL (animals fed westernized diet and gavage with saline); WGOAT (animals fed westernized diet and supplementation with GMW). Each group of pups was increased of p letter (pups at 23 days old) or p2 letter (pups at 45 days old) for identification of age of experiment in the offspring. The chemical composition of diets used in the study are presented in the Table 2.

From the pre-established exclusion criterion (in order to avoid the influence of birth weight on body weight ponderal evolution), only pups weighing > 6g and lesser <7g on the first day postpartum were included in the study (in order to avoid the influence of delivery weight on weigh able evolution). At delivery, each litter were formed with 8 animals/each and four or five males:female. At weaning, only three or four males from each litter were maintained in the study, receiving the same treatment applied to the dams. Six animals from each group were subsequently euthanized at weaning (23 days old), which formed the first phase of the experiment. The other animals submitted the same initial treatment were followed up to 45 days of life, which formed the second phase of the experiment. After 23 old days of age the pups of each group of the second phase of experiment (control or western) also received by gavage goat milk whey until euthanasia´s day.The groups were initially randomized for the first stage of the experiment (31 altogether) and were referred to as CSALp (n = 8), CGOATp (n = 7), WSALp (n = 8) and WGOATp (n = 8), but there were some deaths when gavage was performed and only 6 remained in each of the groups until the day of the euthanasia. The groups for the second phase of the experiment (39 animals) were referred to as CSALp2 (n = 9), CGOATp2 (n = 10), WSALp2 (n = 10) and WGOATp2 (n = 10). The experimental design of the study is shown in Figure 1.

In dams, the GMW was used as supplement forming the control (CGOAT) and westernized groups (WGOAT).

The solution (GMW) was diluted in saline (0.1 g/mL) [21] and administered by gavage (1g/kg body weight) from the day of pregnancy detection onward. Likewise, gavage was performed in CSAL and WSAL groups with saline (0.9 g/100g) at 10 mL/kg animal body weight.

Weekly, monitoring of weight gain and food intake of dams and offspring was performed on the same day and time. The animal daily´s food consumption was determined by the difference between the amount of food supplied (50 g) at the onset of the light cycle and the amount of food remaining 24 h later. The consumption by animal was calculated weekly.

2.3. Microbial Counts in Feces

The feces of female rats and offspring of the second stage of the experiment were analyzed. Feces samples of rats were collected between the 19th and 21st day of lactation and of offspring in the second stage of the experiment between the 40th and 45th day of the experiment. Feces samples were diluted (1:10) in sterile peptone water and 20-µL aliquots were inoculated, using the microdrop technique, onto Plate Count Agar (Acumedia, USA) followed by anaerobic incubation (Anaerobic System Anaerogen. Oxoid Ltda. Wade Road. UK) at 37 ºC for 48 h for enumerating mesophilic anaerobic bacteria, de Man Rogosa and Sharpe agar (Himedia. India) followed by anaerobic incubation (Anaerobic System Anaerogen, Oxoid Ltda., Wade Road, UK) at 37°C for 72 h for enumerating *Lactobacillus* spp., Bifidobacterium agar (Himedia. India), followed by anaerobic incubation (Anaerobic System Anaerogen, Oxoid Ltda., Wade Road, UK) at 37°C for 72 h for enumerating *Bifidobacterium* spp., in MacConkey agar (Acumedia, USA), further followed by aerobic incubation at 37°C for 48 h for enumerating Enterobacteriaceae, and BBE agar (Acumedia, USA) followed by anaerobic incubation (Anaerobic System Anaerogen, Oxoid Ltda., Wade Road, UK) at 37°C for 72 h for enumerating *Bacteroides* spp. [19]. After the incubation period, the colony forming unities (CFU) were counted and the results expressed as $CFU.g^{-1}$.
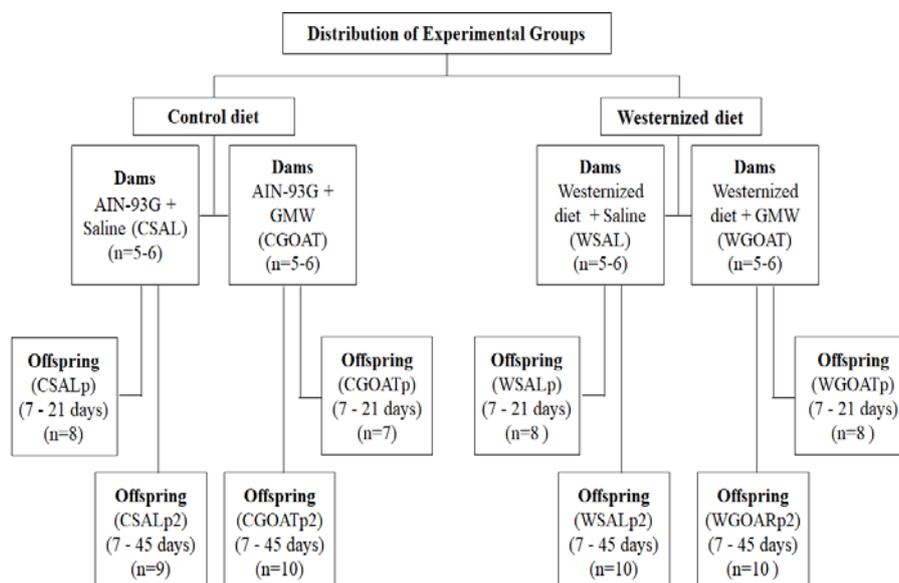

Figure 1. Experimental study design

2.4. Murinometric Parameters

Animals were anesthetized and their body weight, body length (naso-anal), abdominal circumference (AC) and chest circumference (CC) were measured before euthanasia, and the body mass index (BMI) determined [22].

2.5. Euthanasia

After the experimental period, animals were fasted for 12h and intraperitoneally anaesthetized (i.p.) with 45 mg/kg ketamine hydrochloride and 10 mg/kg of xylazine hydrochloride.

At weaning, female rats and eight offspring from each group were euthanized by cardiac puncture. Remaining offspring (ten from each experimental group) were euthanized on the 44th day after birth.

2.6. Tissue´s Weighing and Histological Analysis of the Intestine

Abdominal adipose tissue, liver and intestine were collected and weighed to obtain the relative weight expression. The relative weight calculated in g/10 g body weight was obtained for offspring on the 23rd day during the first part of study. For dams and offspring of 45 days old (second part of study), the relative body weight was calculated in g/100 g. The intestinal portion of jejunum was cleaned (0.9 g/100mL NaCl) and conditioned in formalin fixative solution buffered at 10%. The material was subsequently embedded in paraffin, cut at 5 μm thickness according to the same cross-sectional plane to the intestine. Processed slides were stained with hematoxylin-eosin (HE) and submitted for analysis under optical microscope (Motic BA 200) in increasing objectives and photographed using the 40x objective. The presence of intestinal inflammatory patterns such as stasis, cell migration, hemorrhage, vasodilatation, necrosis, epithelial preservation, hypertrophy and hyperplasia of the smooth muscle layer were evaluated [23].
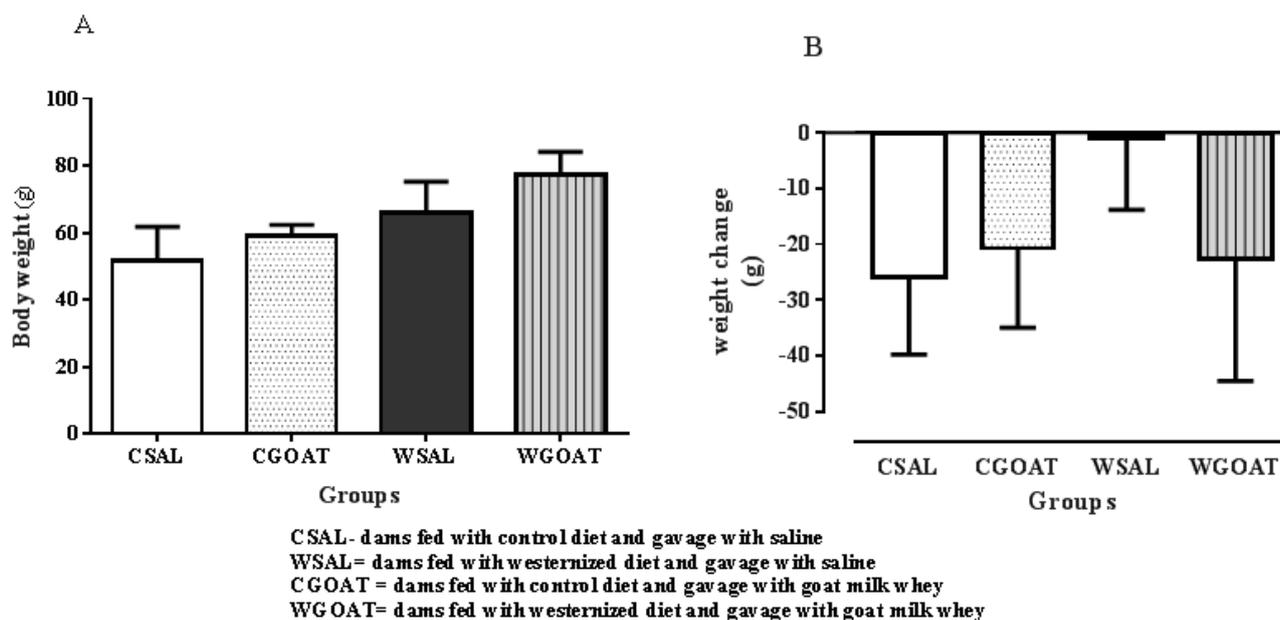
2.7. Statistical Analysis

The Kolmogorov-Smirnov test was used to evaluate normal data distribution. Two-way repeated measures ANOVA was used to analyze the average differences between food intake and weight gain according to diet and GMW supplementation, as well as the interaction between diet and GMW supplementation versus time for each of the parameters. One-way ANOVA was used for analysis of wet weight of organs, BMI and weight gain, while the Tukey´s test (one-way ANOVA) and Bonferroni´s test (two-way RM ANOVA) post-hoc were used to test for multiple comparisons between groups. Significance was set at $p < 0.05$ in all tests. The westernized supplemented group (WGOAT) or control supplemented (CGOAT) group were compared to its control peer (WSAL or CSAL, respectively). The comparisons also were realized between groups with same supplementation and different dietetic treatment (WSAL vs CSAL and WGOAT vs CGOAT).

Values are present as mean ± standard deviation. Statistical analysis and preparation of figures was performed using the GraphPad Prism 6.0 software (GraphPad Software Inc., La Jolla, CA, USA).
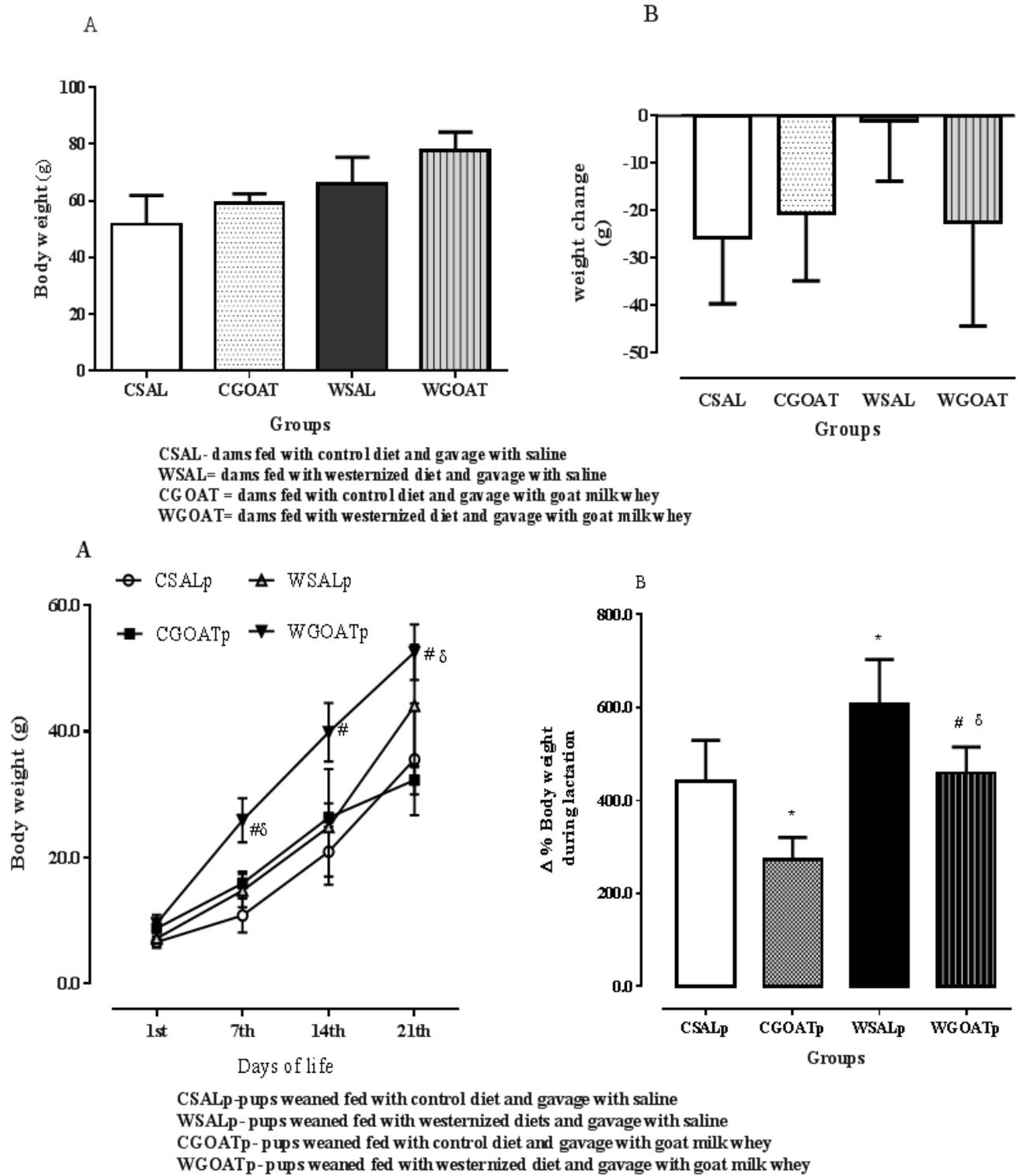
3. Results

3.1. Body Weight and Food Intake of dams AND Pups

The average body weight of female rats at baseline did not differ among groups (p> 0.05). The body weight of female rats of control group were 248.7 ± 16.0 g and of the westernized group were 248.8± 14.4 g. The weight gain during pregnancy (Figure 2A) and weight variation during lactation (Figure 2B) showed no significant differences (p> 0.05) in dams.

CSAL- dams fed with control diet and gavage with saline
WSAL= dams fed with westernized diet and gavage with saline
CGOAT = dams fed with control diet and gavage with goat milk whey
WGOAT= dams fed with westernized diet and gavage with goat milk whey

Figure 2. Body weight (A) and body weight variation (Δ%) in dams (B) from groups that consumed control diet or westernized during pregnancy (A) and lactation (B). Data expressed as mean and standard deviation (SD). One-way ANOVA test

A

B

CSAL- dams fed with control diet and gavage with saline
WSAL= dams fed with westernized diet and gavage with saline
CGOAT = dams fed with control diet and gavage with goat milk whey
WGOAT= dams fed with westernized diet and gavage with goat milk whey

A

B

CSALp -pups weaned fed with control diet and gavage with saline
WSALp- pups weaned fed with westernized diets and gavage with saline
CGOATp- pups weaned fed with control diet and gavage with goat milk whey
WGOATp- pups weaned fed with westernized diet and gavage with goat milk whey

Figure 3. Weight of offspring during lactation (2A) and after weaning (2C), weight gain variation (Δ%) during lactation (2B) and after weaning (2D). Data presented as mean ± SD. Two-way RM ANOVA test (A, C) and one-way ANOVA test (B, D) followed by Tukey post-hoc test. * p <0.05 vs CSAL; # vs. CGOAT; δvs WSAL

The offspring of the first stage from dams who consumed westernized diet showed higher body weight (p <0.05) at the end of lactation (Figure 3A) (Figure 3B). Therefore, there was a significant interaction between time [F (9.87) = 8.83, p <0.00) and dietary treatment [F (3.87) = 413.69. p <0.00]; and interaction between diet and time [F (29.87) = 3.13. p <0.00]. The supplementation with GMW reduced weight gain in both groups during the second stage of the experiment (Figure 2C), showing only inter-group differences (p <0.05); i.e. between CSALp2

and WSALp2 and between CGOATp2 and WGOATp2 (Figure 3D).

Diet consumption during pregnancy (Figure 4) only showed significant differences in the second week, with an effect of treatment time [F (2.57) = 4.71. p = 0.01] and dietary treatment being observed among groups [F (3.57) = 13.62. p = 0.00]. Thus, an interaction between treatment time and dietary treatment was observed [F (6.57) = 0.98. p = 0.013]. There was a significant effect of treatment time on diet intake during lactation

[F (2.54) = 9.20)], but not of dietary treatment among groups [F (3.54) = 0.26. p = 0.086)] (Figure 4). Despite the 18% difference of energy intake (kcal) in the westernized diet, the energetic consumption results showed no differences among groups (p> 0.05).
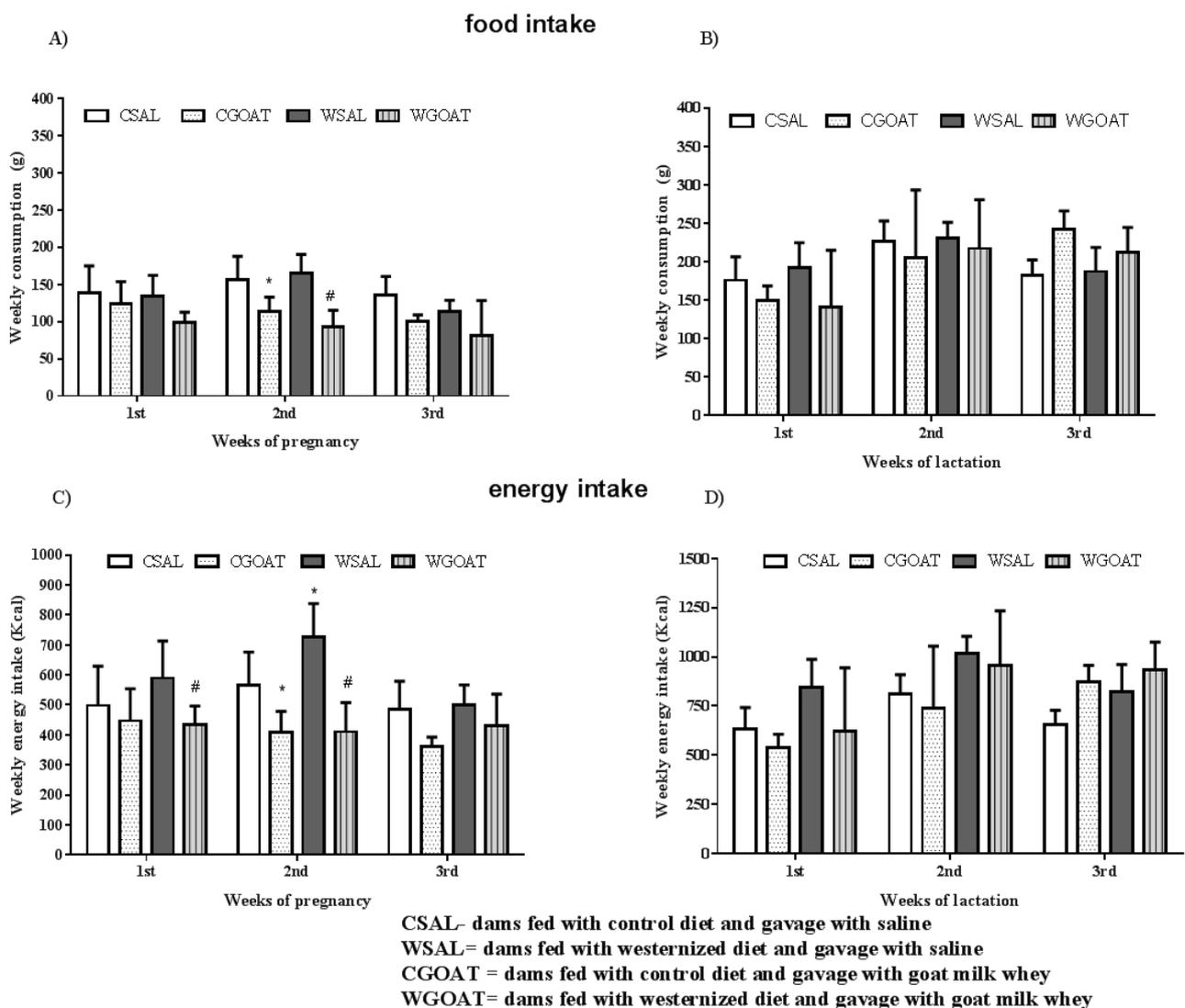
The cumulative food intake of offspring after weaning showed that supplementation with GMW did not modify food intake in control groups (CSALp2=192.8±26.4g; CGOATp2=196.7±21.7g) but changed the westernized diet consumption when WGOATp2 showed higher intake than WSALp2 group (WGOATp2=187.9±6.5g; WSALp2 =152.1±12.2g. p<0.05). Considering the energy consumption, WGOATp2 group consumed more energy than WSALp2 and CGOATp2 groups (WGOATp2= 826.8±28.5kcal; WSALp2= 669.2±53.6kcal; CGOATp2= 727.7±80.4kcal; CSAL=713.3±97.5kcal, p< 0.05).

3.2. Murinometric Parameters and Weight of Organs and Tissues

The murinometric parameters and weight of organs measured at the end of the lactation period were similar among groups (p< 0.05). Regarding offspring of the first phase of the experiment (23 days), the groups fed the westernized diet had higher BMI, CC and AC parameters (p <0.05) despite GMW supplementation compared to their respective control groups fed the control diet (Table 3). As for the relative weight of organs, abdominal adipose tissue was higher in WSALp group compared to CSALp and in WGOATp group compared to CGOATp group; however, no difference was observed in wet relative weight (g/100g) of liver or intestine (Table 3).

In the second stage of the experiment (pups at 45 days old), the group fed the westernized diet and GMW showed lower BMI and CC, and WSALp2 group showed higher BMI compared to the other experimental groups (p <0.05). CC was also lower in CGOATp2 group compared to all experimental groups. Regarding the weight of organs, the supplementation with GMW increased liver weight in both groups receiving supplementation, regardless of the consumed diet. Abdominal adipose tissue was higher in WSALp2 group compared to CSALp2 control group, but no differences were found among groups receiving GMW supplementation. The WGOATp2 group showed greater intestine weight compared to CGOATp2 and WSALp2.

CSAL– dams fed with control diet and gavage with saline
WSAL= dams fed with westernized diet and gavage with saline
CGOAT = dams fed with control diet and gavage with goat milk whey
WGOAT= dams fed with westernized diet and gavage with goat milk whey

Figure 4. Influence of maternal diet and supplementation with goat milk whey on food intake of dams during the weeks of pregnancy (A) and lactation (B) and on the energy value during pregnancy (C) and lactation (D). Data are expressed as mean ± SD. results from the Two Way RM ANOVA test followed by Bonferroni post-hoc-test. * P <0.05 vs CSAL; # p <0.05 vs. WSAL

Table 3. Murinometric parameters and relative weight of organs (g%) of dams and offspring in the 1st (23 day old) and 2nd stages (45 days old) of the experiment
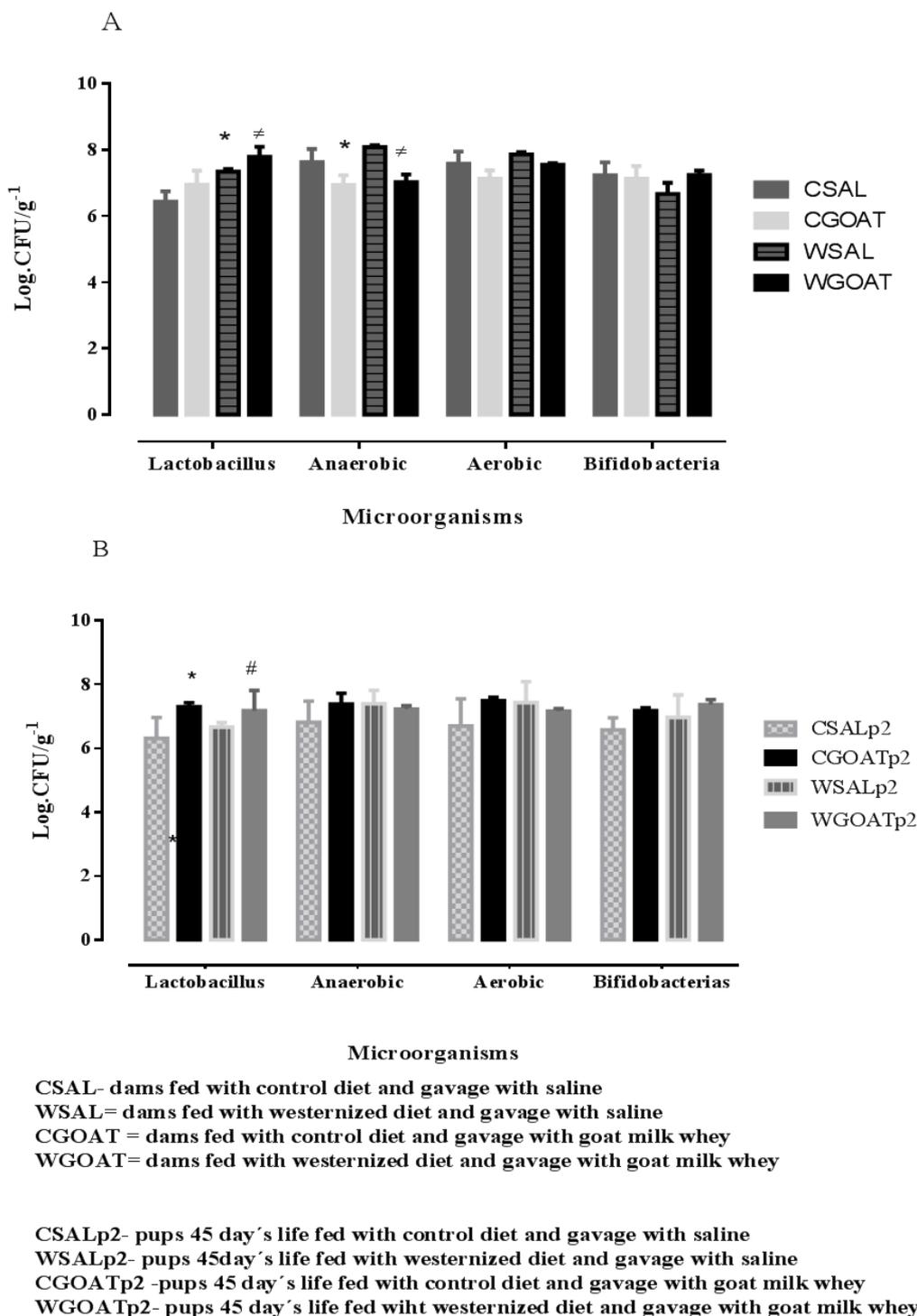
VARIABLE	GROUPS				
Female rats	CSAL (n=6)	CGOAT (n=6)	WSAL (n=6)	WGOAT (n=6)	P value
Murinometric parameters					
BMI (cm/g^2)	0.46±0.05	0.42±0.03	0.48±0.05	0.46±0.03	=0.10
CC (cm)	12.25±0.61	11.50±0.45	12.60±0.42	12.08±0.80	=0.06
AC (cm)	13.83±1.13	12.33±0.98	13.80±1.15	13.42±0.92	=0.08
Body weight	236.67±34.45	203.33±21.37	249.00±31.30	220.00±20.25	=0.06
Organ weights (g/100g of body weight)					
Abdominal fat	2.99±1.21	1.95±0.80	1.50±0.89	0.78±0.10*	<0.05
Liver	3.66±0.45	3.79±0.57	3.38±0.36	3.72±0.43	=0.44
Gut	4.76±0.62	3.92±0.75	4.77±0.71	4.73±0.54	=0.10
Offspring 23d	CSALp (n=8)	CGOATp (n=7)	WSALp (n=8)	WGOATp (n=8)	P value
Murinometric parameters					
BMI (cm/g^2)	0.25±0.04	0.27±0.03	0.31±0.04*	0.29±0.04	<0.00
CC (cm)	7.56±0.68	7.07±0.53	8.55±0.50*	8.63±0.74#	< 0.00
AC (cm)	8.19±0.70	7.21±0.64	8.87±0.55	8.94±0.82#	< 0.00
Body weight	35.01±9.43	32.36±2.29	49.75±4.34*	51.50±6.63#	< 0.00
Organ weights (g/10 g of weight)					
Abdominal fat	0.04±0.01	0.05±0.01	0.12±0.03*	0.09±0.03#	<0.00
Liver	0.37±0.10	0.38±0.02	0.33±0.04	0.36±0.01	=0.38
Gut	0.56±0.15	0.46±0.04	0.57±0.17	0.53±0.08	=0.40
Offspring 45d	CSALp$_2$ (n=9)	CGOATp$_2$ (n=10)	WSALp$_2$ (n=10)	WGOATp$_2$ (n=10)	P value
Murinometric parameters					
BMI (cm/g^2)	0.40±0.03	0.34±0.04	0.47±0.04 *$^\delta$	0.38±0.06	<0.00
CC (cm)	10.56±0.73	9.78±0.75 *$^\delta$	11.05±0.60	10.45±0.76	<0.00
AC (cm)	12.0±0.83	11.39±0.93	12.15±1.20	11.85±1.27	=0.47
Body weight	122.78±1679	96.50±14.82 *	156.00±20.39*	120.50±24.55&	<0.00
Organ weights (g/100g de peso)					
Abdominal fat	2.23±0.41	2.58±0.63	3.46±0.79*	3.23±1.05	<0.05
Liver	3.77±0.32	4.90±0.96*	3.96±0.28	5.12±1.18&	<0.00
Gut	4.57±0.74	5.47±1.25	5.35±0.67	7.66±1.33#&	<0.00

CSAL = dams fed with control diet and gavage with saline; CGOAT = dams fed with control diet supplemented with goat milk whey; WSAL = dams fed with westernized diet and gavage with saline; WGOAT= dams fed with westernized diet supplemented with goat milk whey Data are expressed as mean ± SD. results obtained from the one-way ANOVA test followed by Tukey post-hoc test. *p<0.05 vs CSAL; #p<0.05 vs CGOAT; &p<0.05 vs WSAL; δvs WGOAT. AC = abdominal circumference. CC = chest circumference; BMI = body mass index.

3.3. Fecal Microbial Composition

Supplementation with GMW in female rats during pregnancy and lactation (Figure 5A) did not cause differences in *Lactobacillus* spp. counts in intra-group evaluation in controls (CSAL=6.43±0.35 log CFU/g^{-1}; CGOAT=6.94±0.49 log CFU.g^{-1}), as well as among groups fed the westernized diet (WSAL=7.34±0.09 log CFU.g^{-1}; WGOAT=7.78±0.35 log CFU.g^{-1}). However, groups receiving GMW supplementation showed higher *Lactobacillus* spp. counts compared to their respective control groups (WSAL=7.34±0.08 log CFU.g^{-1}; CSAL=6.43±0.31 log CFU.g^{-1}; WGOAT= 7.79±0.30 log CFU.g^{-1}; CGOAT=6.94±0.45 log CFU.g^{-1}). For the mesophilic aerobic bacteria counts, the GMW supplementation caused decreased counts in both the control group (CSAL= 7.58±0.37 log CFU.g^{-1}; CGOAT= 6.94±0.29 log CFU / g^{-1}) and the group fed the westernized diet (WSAL= 8.08±0.06 log CFU.g^{-1};

WGOAT= 7.02±0.23 log CFU.g^{-1}). No differences among groups were observed for the *Bifidobacterium* spp. and total anaerobic counts. The offspring (Figure 5B) showed no differences (p <0.05) in counts of the monitored microbial groups in feces, with the exception of *Lactobacillus* spp. counts which were higher in groups receiving GMW supplementation compared to their respective control groups (CSALp2 =6.31±0.57 log CFU.g^{-1}; CGOATp2=7.30±0.11 log CFU.g^{-1}; WSALp2= 6.66±0.14 log CFU.g^{-1}; WGOATp2=7.40±0.29 log CFU.g^{-1}) (Figure 5). For the offspring at 45 days, the WSALp2 group showed lower (~0.92 log cycles) Enterobacteriaceae counts compared to CSALp2 and WGOATp2 groups (WSALp2=6.85±0.26 log CFU.g^{-1}; CSALp2=7.77±0.35 log CFU.g^{-1}; WGOATp2=7.76±0.41 log CFU.g^{-1}). The WGOATp2 group presented bigger Bacteroides spp. counts than WSALp2 group (WSALp2 = 7.15±0.1210 log CFU.g^{-1}; WGOATp2= 7.62±0.10 log CFU.g^{-1}).

A

B

CSAL- dams fed with control diet and gavage with saline
WSAL= dams fed with westernized diet and gavage with saline
CGOAT = dams fed with control diet and gavage with goat milk whey
WGOAT= dams fed with westernized diet and gavage with goat milk whey

CSALp2- pups 45 day´s life fed with control diet and gavage with saline
WSALp2- pups 45day´s life fed with westernized diet and gavage with saline
CGOATp2 -pups 45 day´s life fed with control diet and gavage with goat milk whey
WGOATp2- pups 45 day´s life fed wiht westernized diet and gavage with goat milk whey

Figure 5. Mean populations of microorganisms in feces samples grown in aerobic (aerobic bacteria) and anaerobic conditions (Lactobacillus, Bifidobacteria and mesophilic anaerobic bacteria) of dams (A) and offspring (B) fed with control or westernized diet and treated with saline or goat milk whey. One-way ANOVA test followed by Tukey´s post-hoc test. Data expressed in Log10 of the number of colony-forming units / ml. * P <0.05 vs CSAL and #vs CGOAT

3.4. Histological Analysis of the Intestine

Morphological evaluation was performed by histological analysis of intestinal cells, particularly in jejunum cells (Figure 6A). Cells from groups fed the westernized diet (WSAL) presented epithelial wear, destruction (triangle) and necrosis (arrow) of villi and central hemorrhage with a loss in tissue architecture (Figure 6A). No changes in cell morphology were detected in control groups (CSAL and CGOAT) (Figure 6A). However, rats fed the westernized diet and supplemented with GMW (WGOAT) showed an absence of inflammation resembling animals from the control group (Figure 6A).

Similar results were found for offspring at 23 days old (Figure 6B) when the WSALp group showed inflammation (star) and epithelial destruction (arrow). However, offspring fed the westernized diet for a longer period showed worsening of epithelial wear and villi destruction. Supplementation with GMW (CGOATp2 and WGOATp2) also attenuated these adverse effects with absence of inflammation (Figure 6C).
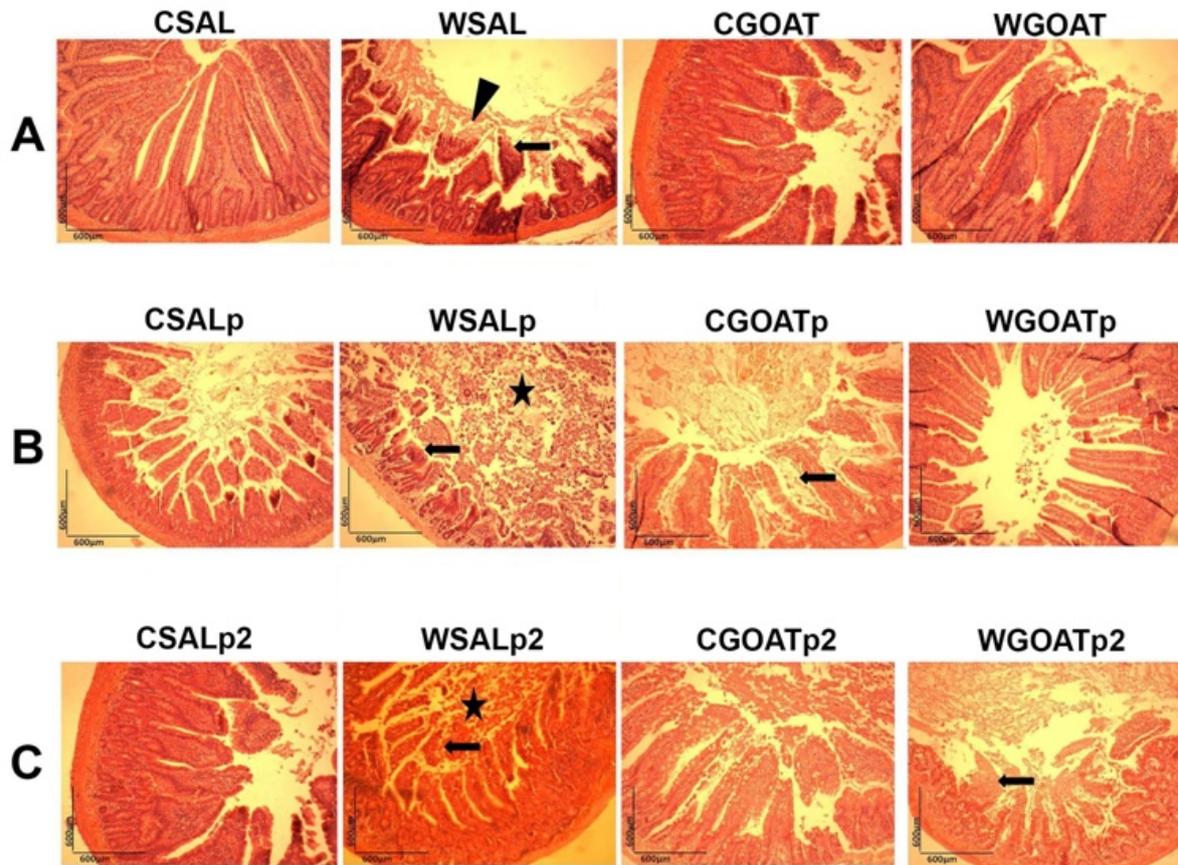
Figure 6. Morphology of jejunum of dams (A), offspring at 23 days (B) and at 45 days old (C). Microscopy image with 100x magnification. CSAL: dams fed with control diet and gavage with saline; WSAL = dams fed with westernized diet and gavage with saline; CGOAT = dams fed with control diet and supplementation with goat milk whey; WGOAT = dams fed with westernized diet and supplementation with goat milk whey. The offspring acronyms at 23 days old are added of letter p and offspring acronyms at 45 days old are added of letter p2. Arrow: epithelial destruction. Star: inflammatory exudate. Triangle: necrosis. Resolution 40X

The westernized diet and GMW offered to female rats and offspring influenced several of the evaluated parameters. In female rats, weight changes, murinometric parameters and food intake were affected by diet and GMW supplementation. Increase in murinometric measures and body weight during lactation was observed in offspring fed the westernized diet. The continuity of GMW supplementation to offspring until the end of childhood promoted a reduction in weight gain and murinometric measures in both groups receiving supplementation with GMW, independent of the type of diet ingested.

The body weight and food intake results observed for dams and offspring corroborate the results observed by a previous study using a similar westernized diet [24]. A lower weight gain was also observed in offspring fed cow milk whey derived protein compared to those fed casein protein [25]. Whey protein derived peptides were also able to modulate the body weight gain and intestinal microbiota colonization in mice [26].

Higher counts of *Lactobacillus* spp. were observed in offspring receiving GMW supplementation compared to non-supplemented peers. The set of results suggests that the diet could interfere in microbial colonization, and this in turn probably affects body weight gain through mechanisms associated with synthesis of hormones involved in energy homeostasis and body fat storage [27]. Interestingly, the whey protein hydrolysate had been suggested attenuated of loss of lean muscle mass [28] or

modulate the bodyweight by influence in the satiety [29]. Thus, if western-style diet increases the body weight, the supplementation of goat whey milk may have beneficial effects by control body weight gain. However, more than total body weight, the ectopic fat, abdominal or visceral fat, is considered one of the most deleterious events in the appearance of metabolic disorders. Our results show that the westernized diet promoted greater abdominal fat deposit in offspring at 23 days. Nevertheless, the continuity of offspring being fed the westernized diet with added supplementation of GMW abolished the significance found at day 23rd in WGOATp2 offspring. The magnitude of body fat difference reached 55% more in WSALp2 compared to CSALp2. The effect of the westernized diet on the increased abdominal fat amounts in offspring corroborates the findings of previous studies [5,24,30]. The increase in visceral fat observed early in offspring fed the westernized diet remained at 45 days old, but there was a lack of significance in the group supplemented with GMW, revealing a positive effect of GMW supplementation in this group. However, not only diet composition, but also the supplementation period may interfere in this response because increased visceral fat was not observed in the control group receiving GMW supplementation. Although previous study have found that the decrease of visceral fat in offspring was higher when the supplementation with oligosaccharides derived from GMW was administered only during gestation [31].

The highest liver weight in CGOATp2 and WGOATp2 was probably due to the GMW supplementation and not only to the westernized diet ingestion. Previous studies have verified no increase in the liver weight of rats fed a westernized diet during the perinatal period [30,32]. The steatosis non-alcoholic fatty liver disease (NAFLD) is defined as pathologic accumulation of fat in the form of triglycerides, which when associated with cellular injury and inflammation coupled with the excessive fat accumulation is referred to as non-alcoholic steatohepatitis-NASH [33]. A previous study also observed hepatic steatosis in rats fed with goat's milk rich in conjugated linoleic acid [31], but this effect still causes controversial results among researches [34,35,36]. The NAFLD is a disease associated with dietary fat intake independent of excess caloric consumption [37] and often associated with the most common clinical features of metabolic syndrome, such as central obesity, type 2 diabetes mellitus, dyslipidemia and arterial hypertension [38] reaching prevalence in the general population of Western countries ranges from 25% to 30% [39].

A westernized diet increases the bacteria counts belonging to phylum Firmicutes which are associated with negative impacts on human health [40]. However, our results show that GMW supplementation increases the number of total lactobacilli in both dams and offspring. Mice fed a westernized diet with added cow milk whey presented increased *Lactobacillus* spp. and *Bifidobacterium* spp. fecal counts [11]. Similarly, consuming an isolated fraction of oligosaccharides from GMW increased the fecal *Lactobacillus* spp. and *Bifidobacterium* spp. counts in rats [41]. Oligosaccharides are present in higher amounts in goat milk than in bovine milk; however, goat milk presents similar oligosaccharide composition to human milk. Additionally, goat milk was capable of reducing inflammatory process in individuals fed obesogenic diets and with increased body fat [14]. More recently was observed in newly weaned rat reduction in the amount of Clostridium perfringens in intestine and significant anti-inflammatory properties when used goat and cow milk powders isolated or combined with prebiotic in diet [42].

Although the total anaerobic bacteria count remained unchanged, offspring fed the westernized diet and receiving GMW supplementation at 45 days old presented increased Enterobacteriaceae and Bacteroides spp counts. Reduced anaerobic bacteria counts were observed in obese humans, indicating a relationship between high Enterobacteriacea and Bacteroides spp counts in gut microbiome and obesity occurrence [43].

A previous study observed that the ingestion of a westernized diet was capable of altering intestinal morphology by releasing pro-inflammatory cytokines (IL-6, IL-1β, TNF-α and KC) through the intestinal mucosa [44]. In addition, lesions and infiltrated inflammatory cells have been observed in dams and offspring [4]. Our results demonstrate that GMW supplementation may minimize the adverse outcomes on visceral fat and intestinal morphology caused by a westernized diet. This effect was observed in recent study utilizing goat milk powder in diet of newly weaned rats [42] presence of oligosaccharides in GMW, which already have shown to possess prebiotic and anti-inflammatory

effects [11]. Additional studies may consider the hypothesis that bioactive compounds present in goat's whey can be applied to the amelioration alterations on intestinal morphology and modulate the microbial counts in feces of rats submitted to a westernized diet from gestation.

In conclusion, the results of this study show that the GMW supplementation during pregnancy and lactation induce positive impacts on intestinal morphology aspects, with reduction of inflammatory processes, and microbial counts, higher count for the group *Lactobacillus* spp. compared to their respective control groups, in feces of dams fed a westernized diet. In offspring, the beneficial effects of GMW supplementation on intestinal morphology, microbial counts in feces, body weight modulation and abdominal fat changes varied with both the type of diet and the age investigated (23rd or 45th day after birth). In general, pups at 23 and 45 days of age, showed reduction of inflammatory processes in the small intestine, higher counts of *Lactobacillus* spp. in the feces, reduction of weight gain as well as abdominal fat. In set, these results indicate the potential use of GMW such as dietary supplement to protect the harmful effects caused by westernized diet consumption on nutritional status, fecal microbial composition and intestinal health.

Disclosure of Interest

Abbreviations

ANOVA, Analysis of Variance; AIN, American Institute of Nutrition; CONCEA, National Council for Animal Experimentation Control; CSAL, Control Diet and Gavage with Saline; CGOAT, Control Diet Supplemented with Goat Milk Whey; BMI, Body Mass Index; CFU, Colony Forming Unit; UFPB, Federal University of Paraíba; UFPE, Federal University of Pernambuco; GMW, Goat Milk Whey; WSAL, Westernized Diet and Gavage with Saline; WGOAT, Westernized Diet Supplemented with Goat Milk Whey.

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