

Effect of Chronic Alcohol Consumption on Nutritional Composition of Breast Milk: An Experimental Study

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Received November 11, 2018; Revised December 16, 2018; Accepted December 26, 2018

Abstract The aim of the present study was to analyze the nutritional effects of alcohol consumption in the form of distilled and fermented beverages, together with a diet made up of habitual foods of northeastern Brazil, on the composition of breast milk. Sixty Wistar rats were divided into five experimental groups, and all received a balanced diet of beans, rice, chicken, cassava flour and soybean oil (18% protein content), associated to different liquids in accordance with the following experimental groups: Control Group (CG) – distilled water; Alcohol Group (AG) – 5% hydroalcoholic solution; Peer-Fed Alcohol Group (PFAg) – maltodextrin solution replacing calories from alcohol; Beer Group (BG) – beer (5% ethanol v/v); Peer-Fed Beer Group (PFBG) - maltodextrin solution replacing calories from beer. On the 12th day of lactation, breast milk was withdrawn for the analysis of protein, carbohydrates, total lipids, fraction of triglyceride, sodium and potassium. The Mann-Whitney test was used for the statistical analysis, with the level of significance set at 5% ($p < 0.05$). There was an increase in potassium content in the breast milk in the AG and an increase in lactose content in the breast milk in the BG. It may be concluded that the use of alcoholic beverages, even those with low ethanol content, during lactation can cause serious alterations in the metabolism of mothers, altering the composition of breast milk and possibly compromising the normal development of newborns

Keywords: alcohol drinking, lactation, breast milk, nutritional composition, animal models

Cite This Article: C.K.R. Monte-Guedes, A.B.M. Filho, A. F. L. M. Guedes, B. M. Padilha, K.S. Batista, J.S. Aquino, and M.G.P.A. Burgos, "Effect of Chronic Alcohol Consumption on Nutritional Composition of Breast Milk: An Experimental Study." *Journal of Food and Nutrition Research*, vol. 6, no. 12 (2018): 767-772. doi: 10.12691/jfmr-6-12-7.

1. Introduction

A number of studies have described the effect of the maternal consumption of alcoholic beverages on the growth and development of embryos [1,2]. Serious alterations include miscarriage and fetal alcohol syndrome, which is characterized by a specific pattern of malformations in children born of mothers who drink [3]. However, references on the effects of alcohol intake on lactation and possible alterations in milk production and breastfeeding are little known [4].

Due to its solubility, low molecular weight and the fact that it does not undergo ionization, alcohol is easily passed to breast milk, where its concentration is directly proportional to that in the blood. Thus, as the drug reaches the blood, it accumulates in the breast milk [4,5].

Ethanol induces the formation of oxygen free radicals, which can damage cellular proteins and lipids, thereby increasing apoptosis and hindering adequate development [6], although some findings indicate that alcoholic beverages

enhance milk production, especially when ingested prior to breastfeeding, facilitate its withdrawal and strengthen the mother-child interaction [7,8,9].

A recent study carried out in Australia on the prevalence of maternal alcohol consumption during breastfeeding found that 43% of nursing mothers had consumed alcohol in the previous 12 months [10]. A study carried out in the early 1920s in Brazil revealed a high percentage (84%) of medical prescriptions of beer as a milk-producing agent and that the practice was adopted by approximately 76% of the nursing mothers interviewed [7]. A study carried out in the city of Sao Paulo in the 1990s revealed that many nursing mothers consider dark beer to be a stimulator of breast milk [11]. Moreover, a study carried out with 40 nursing mothers found that 45% had been counseled by their physicians and/or nurses to consume alcohol during lactation and 35% received the same advice from family members and friends [12].

The National Institute of Child Health and Human Development in the United States found significant differences in the motor development of newborns of mothers who regularly ingested alcoholic beverages [13].

A number of authors report diminished weight and stature [1,14] as well as an increase in neonatal mortality [15]. Moreover, maternal metabolic and hormonal alterations are reported when alcohol is ingested during the breastfeeding period, leading to alterations in the composition of breast milk that compromise the adequate transmission of nutrients to offspring, with significant repercussions on growth and development [4,16,17].

Recently reports deny the hypothesis that breastfeeding is benefited by the consumption of alcohol [18,19].

The effects of alcohol intake on the composition of breast milk have not been sufficiently studied to the point of clarifying all the controversial issues related to this habit. Thus, experimental studies on rats aimed at assessing the effects of the consumption of alcoholic beverages in combination with regional foods on the nutritional quality of breast milk can contribute further knowledge on this subject. Moreover, the findings of such studies could serve healthcare professionals with regard to adequate clinical-nutritional orientation for nursing mothers.

Thus, the aim of the present study was to determine the composition of the breast milk of rats submitted to ethanol intake in the form of distilled and fermented beverages in combination with a blend of regional foods consumed in northeastern Brazil.

2. Methods

2.1. Animals and Experimental Conditions

Sixty female Wistar rats (*Rattus Norvegicus*, Albinus variety) aged 100 to 120 days and weighing 260 to 360 g were randomly assigned and maintained under standard laboratory conditions, with a 12-h light/dark photoperiod, constant temperature of $23^{\circ} \pm 1^{\circ} \text{C}$ and relative humidity of $55\% \pm 10\%$. The rats were mated with males of the same variety at a proportion of one male to three females for five days, with a two-day rest period followed by mating for an additional five days (totaling 12 days), in compliance with the norms established for the lineage.

This study received approval from the Ethics Committee for Animal Experimentation of the Center for Biological Sciences of the Universidade Federal de Pernambuco (process: 097/2002).

2.2. Experimental Design

After the birth of the offspring, the animals were divided into five groups of 12 animals each. The Control Group (CG) received water and a balanced diet (18% protein) for lactation composed of a blend of foods offered for consumption ad libitum. The Alcohol Group (AG) received a hydroalcoholic solution containing 5% ethanol and the control diet ad libitum. The Peer-Fed Alcohol Group (PFAG) received a water and maltodextrin solution and the control diet with the same caloric proportions ingested from the liquids in the AG. The Beer Group (BG) received beer (5% ethanol) and the control diet ad libitum. The Peer-Fed Beer Group (PFBG) received a water and maltodextrin solution and the control diet with the same caloric proportions ingested from the liquids in the BG.

The aim of including the two peer-fed groups was to control malnutrition, which is a constant variable in research involving alcohol, especially protein-caloric malnutrition caused by the reduction in food intake resulting from alcohol intake. Thus, the aim of the addition of maltodextrin was to make the liquids isocaloric. This procedure allowed establishing differences between the effects of malnutrition per se and that caused by exclusive alcohol intake.

2.3. Diets and Liquids

The food and beer were acquired during a single trip to the local market in order to ensure that the components of the diets came from the same lot and therefore had the same chemical composition. The control diet was composed of common beans (*Phaseolus vulgaris* L.), processed rice (*Oriza sativa* L.), cassava flour (*Manihot esculenta* Crantz) and chicken (*Gallus galinaceo*). The rice, beans and chicken were cooked separately in water for two hours, dried in an oven (60°C) for 12 hours and ground in a grinder (FLOOR GRIND MILL - CHUO BOEKI KAISHA) for the obtainment of the respective meals. Daily food intake was calculated as the amount offered minus the sum of the non-consumed pellets and residual scraps.

The CG ingested drinking water. The AG ingested a solution of 95 ml of water + 5 ml of ethyl alcohol (P.A. 99.50 purity, MERCK n° 130, producing 7.1 cal/ml). The BG ingested beer (5% ethanol, lot n°066630), made up of water, malt, non-malted cereals, carbohydrates, hops, antioxidant INS 316 and stabilizer INS 405, with a caloric content of 42.9 cal/100 ml. Both peer-fed groups received a water + maltodextrin solution. Table 1 displays the composition of the diet and liquids and Table 2 displays the information on calories.

Table 1. Composition of diet and liquids ingested by animals

Constituents (g)	GROUPS				
	CG	ALCOHOL		BEER	
		AG	PFAG	BG	PFBG
Nutrients (g/100g)					
Rice	34.00	34.00	34.00	34.00	34.00
Beans	30.00	30.00	30.00	30.00	30.00
Cassava flour	12.00	12.00	12.00	12.00	12.00
Chicken	11.00	11.00	11.00	11.00	11.00
Fiber	2.17	2.17	2.17	2.17	2.17
Soybean oil	5.98	5.98	5.98	5.98	5.98
Vitamin blend ¹	1.00	1.00	1.00	1.00	1.00
Minerals ¹	3.50	3.50	3.50	3.50	3.50
Choline bitartrate	0.35	0.35	0.35	0.35	0.35
Alcohol	-	5.00	5.00	-	-
Beer	-	-	-	100.00	100.00
Maltodextrin	-	1.90	8.88	-	10.78
Water	100.00	100.00	100.00	-	-

CG - Control Group; AG - Alcohol Group; PFAG - Peer-Fed Alcohol Group; BG - Beer Group; PFBG - Peer-Fed Beer Group. 1. AIN 93 G (American Institute of Nutrition).

Table 2. Caloric composition of diet and liquids ingested by animals

CONSTITUENTS (g)	GROUPS				
	CG	ALCOHOL		BEER	
		AG	PFAG	BG	PFBG
% of calories ^B					
Protein	18	18	18	18	18
Carbohydrates	64	54	54	54	54
Fat	18	18	18	18	18
Ethanol ^A	-	08	-	-	-
Beer ^A	-	-	-	10	-
Maltodextrin ^A	-	02	10	-	10

CG - Control Group; AG - Alcohol Group; PFAG - Peer-Fed Alcohol Group; BG - Beer Group; PFBG - Peer-Fed Beer Group. A. Calculation of calories: ethanol = 7.1cal/g; beer = 0.43cal/g; maltodextrin = 4.0cal/g; B. 434cal/100 g.

2.4. Weight of Animals

Weight was recorded every two days using an electronic precision scale (OHAUS) with a capacity for 2.6 oz. The change in weight was calculated using weight on the second day as the initial value and weight on the 12th day as the final value.

2.5. Collection of Milk Samples and Sacrifice of Animals

The collection of breast milk was carried out on the 12th day of life of the offspring using the method described by Keen et al. [20]. The mothers were separated from the litter for one to two hours, anesthetized with ketamine (1 ml/g of body weight) in the gluteus muscle and administered an intraperitoneal injection of oxytocin (1 ml = 5UI). After 30 minutes, manual collection of the breast milk was performed through the milking of the teats to withdraw the largest volume of milk. The collection lasted approximately one hour and the milk was placed in 5-ml test tubes, which were immediately stored in a freezer at -20°C for the subsequent determination of carbohydrates, protein, total lipids, triglycerides and minerals. After the collection of the breast milk, the animals were sacrificed through the inhalation of ethyl ether, a central nervous system depressor.

2.6. Determination of Composition of Breast Milk

The analyses for each group were carried out in triplicate and repeated in cases of differences of 10% or greater. The determination of carbohydrates was performed using colorimetric analysis, in which a phenol + concentrated sulfuric acid solution was used for each aliquot of 10 ml de milk, following the method described by Dubbois et al. [21].

The reading of optical density was performed with a spectrophotometer at 540 nm and the results were expressed as g/dl. Total lipids were determined by the colorimetric method with concentrated sulfuric acid, using the Doles commercial kit (Goiânia, GO, Brazil). Absorbance

was read in a spectrophotometer at 530 nm and the results were expressed as mg/dl of milk. Triglycerides (TG) were determined by the enzymatic colorimetric method described by Bucolo and David [22], using the LABORLAB commercial kit (Laborlab Ltda., Guarulhos, SP, Brazil).

Absorbance was read in a spectrophotometer at 505 nm and the results were expressed as mg of TG/dl of milk. Total proteins were determined by the classic biuret method, using the LABORLAB commercial kit, based on the principle of bromocresol-sulfonaphthalein bonding. Absorbance was read in a spectrophotometer at 540 nm and the results were expressed as g/dl of milk. Minerals (sodium and potassium) were analyzed through atomic absorption spectrophotometry [23].

2.7. Statistical Analysis

The databank was constructed and the statistical analysis was performed using the Epi-info 6.04 and SPSS 8.0 programs. Descriptive analysis was used to determine mean and standard error of the mean (SEM) values. The non-parametric Mann-Whitney test was used for the inferential statistical analysis. The choice of the non-parametric test was caused by the number of cases, insufficient to verify a hypothesis of normality of data in each group, what is required for the t-Student test. The level of significance was set at 5% ($p < 0.05$). The Statistical Analysis System (SAS, version 8.0) was used for the statistical calculations.

3. Results

On day 12, the rats in the CG had significantly greater weight values than those in the AG. On the other hand, the rats in the BG had greater weight values than the CG throughout the experimental period, with significant differences on days 2 and 10 of lactation. In the comparison of the AG and BG, a significant weight gain occurred in the latter group beginning on day 2, with this difference maintained on days 4, 8 and 10 (Figure 1).

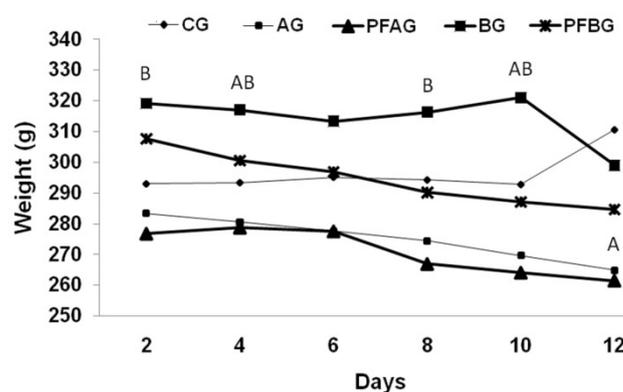


Figure 1. Weight curve of lactating Wistar rats submitted to food blend + beer or ethanol. In figure: CG: Control Group, AG: Alcohol Group, PFAG: Peer Fed Alcohol Group, BG: Beer Group, PFBG: Peer-Fed Beer Group. Markers mean body weight on indicated day. Letters indicate statistically significant difference ($p < 0.05$) between group represented on respective line and group with corresponding letter: A and B to CG and AG, respectively (Mann-Whitney test)

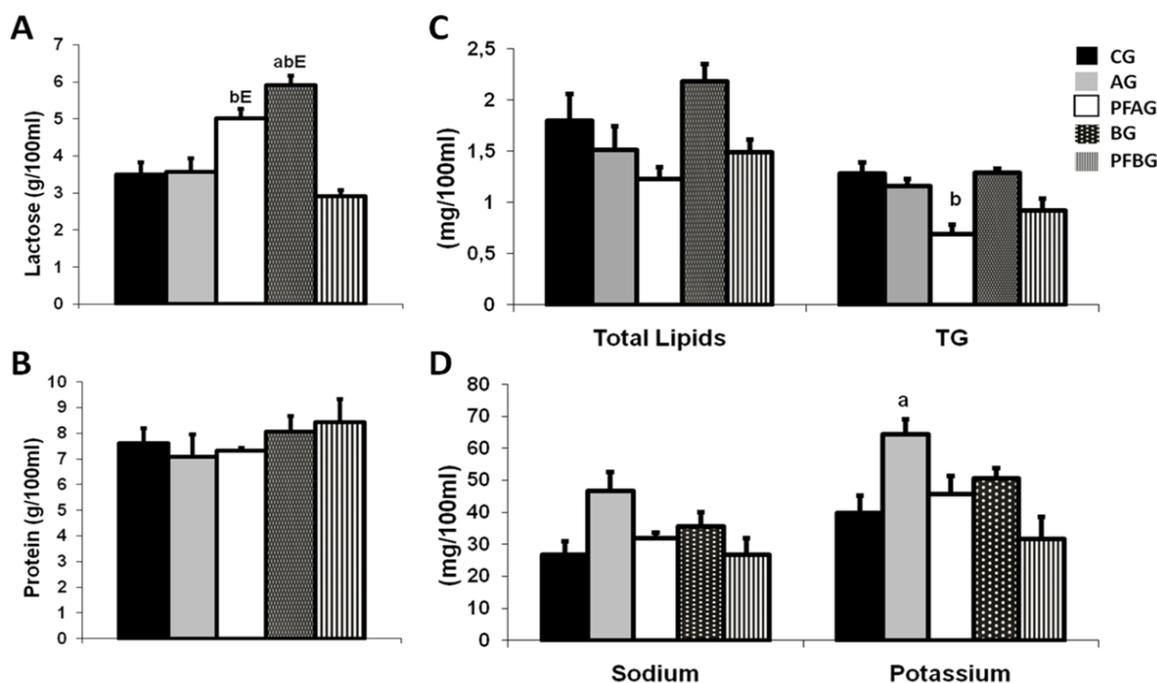


Figure 2. Variation in nutrient content in breast milk of lactating Wistar rats submitted to food blend + beer or ethanol. In figure: A- lactose content, B- protein content, C- lipid and triglyceride fraction content, D - sodium and potassium content. In bars: CG: Control Group, AG: Alcohol Group, PFAG: Peer Fed Alcohol Group, BG: Beer Group, PFBG: Peer-Fed Beer Group. Columns = mean \pm SEM.; different letters indicate statistically significant difference (lowercase = $p < 0.01$, uppercase = $p < 0.05$) between group represented in column and group with respective letter: a, b and E to CG, AG and PFBG, respectively (Mann-Whitney test)

The analysis revealed that both the ingestion of ethanol and beer caused alterations in the composition of breast milk. Figure 2 displays the content in mean \pm SD of lactose (g/100 ml), protein (g/100 ml), total lipids (mg/100 ml), triglyceride fraction (mg/100 ml), sodium (mg/100 ml) and potassium (mg/100 ml) in the different groups.

The PFAG (5.02 ± 0.89) exhibited higher lactose content than the AG (3.57 ± 1.26 ; $p < 0.05$) and PFBG (2.91 ± 0.58 ; $p < 0.01$). The BG (5.92 ± 0.85) exhibited lower lactose content than the CG (3.49 ± 1.14 ; $p < 0.05$), PFAG ($p < 0.05$) and PFBG ($p < 0.01$). The AG and PFBG did not differ significantly from the control.

With regard to the protein content in the breast milk, the CG, AG, PFAG, BG and PFBG respectively exhibited the following mean \pm SD values: 7.61 ± 1.09 , 7.08 ± 3.04 , 7.31 ± 0.44 , 8.06 ± 2.07 and 8.44 ± 3.08 g/100ml. The statistical analysis revealed no significant differences between groups.

The analysis of lipids revealed an interesting difference in TG content, while the total lipid content did not undergo any significant change. The PFAG exhibited 0.69 ± 0.32 mg of TG/100 ml of milk, demonstrating a significant difference in comparison to the AG (1.16 ± 0.23 ; $p < 0.05$).

With regard the mineral content in the breast milk, no statistically significant differences in sodium content were found between groups. Potassium content was significantly higher in the AG (64.48 ± 16.3) in comparison to the CG (39.73 ± 19.27 ; $p < 0.05$).

4. Discussion

The present study employed an alcohol administration method that causes less maternal stress and comes closer

to the human model [24]. According to El-Guindy et al. [25], the administration of alcohol poses one of the greatest difficulties for the comparison of experimental studies due to the wide variety of specific aspects involved. Different studies employ different administration pathways, such as intramuscular, intraperitoneal, liquid diet and gavage, as part of the ad libitum consumption of experimental animals and vapor chambers [1,15,26].

Another basic point discussed in the literature regarding lactating rats is the percentage of alcohol used [1]. The present study used only beverages with 5% ethanol. This alcohol content is considerably lower than values reported in the literature, which often range from 10% to 20%. Two forms of beverage (distilled and fermented) were used to determine whether the alcohol would have the same effect on nutrition and metabolism in the groups studied.

According to Ferner [27], the association between ethanol intake and alcohol blood level is quite variable, depending mainly on the genetic background of the degree of absorption, elimination and volume distributed throughout the body (relationship between body weight and blood concentration). Differences between individuals with regard to this distribution depend on age, gender, ethnicity, height, weight and body composition [16,28].

Murillo-Fuentes et al. [2] and Freitas et al. [26] studying the effects of maternal chronic alcohol administration in the rat, suggest that chronic alcohol administration during lactation affects pup growth, most possible, among others, by alteration on milk consumption and increase of oxidative stress. This can explain the difference of weight gain in CG, AG and BG showed in Figure 1. Pointing to this same explanation, Menella [29] demonstrate that short-term exposure to small amounts of alcohol in mothers' milk produces distinctive changes in the infants' patterns of feeding.

Alcohol has multiple adverse effects on reproduction and lactation, especially due to its role in endocrine functions and secretions [4,9]. Disorders have been documented in the hypothalamus, pituitary gland, ovaries and mammary glands following the consumption of alcohol [17]. Studying 5 human and 15 animal studies examine the possible causal mechanism(s) that may explain the association between alcohol consumption and the risk of developing breast cancer, Oyesanmi et al. [30] report alterations in the structure and function of the breasts.

In the present study, the findings displayed in Figure 2 demonstrate that the fermented beverage (beer) caused changes in the chemical composition of the breast milk, as evidenced by the increase in lactose in the BG. This finding is in agreement with that reported in a study by Sanchis et al. [31], who found an increase in lactose associated to lipid and protein alterations; according to the authors, this finding can be explained by the occurrence of atrophy in the rats who consumed 5% ethanol.

In the present data, the increase in lactose was not accompanied by other macronutrient alterations. This indicates that such an increase was possibly associated to the composition of the beer, which contains malt, non-malted cereals and carbohydrates, and not exclusively to the alcohol content in the beverage.

Lactose content in the CG and AG was within the normal range [32]. This contrasts findings described by Neves et al. [33], who report a reduction in lactose content in rats having received a 20% ethanol solution. However, a 40.6% increase in lactose content was found in the PFAG in relation to the CG.

No similar data or explanation for these findings was found in the literature. However, the reduction in lactose content in the PFBG in relation to the BG was highly significant. This finding could be explained by the fact that this group had the lowest food and total calorie intake, which is in agreement with findings described for malnourished lactating rats in a study carried out by Keen et al. [20].

With regard to fat content, no alteration in quantity was found with the use of ethanol, which is in agreement with the findings of previous studies, although Heil et al. [34] report a change in the profile of fatty acids as well as a reduction in phosphatidylserine. Reduce in TG on PFAG probably is a reflection that the serum lipid profile is a biochemical measure sensitive to nutritional status. It is well reported in literature that lipids and fractions are reduced under malnutrition conditions [35].

About the increase in potassium content in the AG was very likely due to increases potassium entry into the cells in withdrawing alcoholics due to the coexistent respiratory alkalosis in chronic alcohol consumption even the hypophosphatemia been a common electrolyte abnormality observed in alcoholic patients [36].

Finally, in relation to that traditional wisdom claims that moderate beer consumption may be beneficial for initiation of breastfeeding and enhancement of breastfeeding success Koletzko and Lehner [18] report that it is not by alcohol content but apparently to a polysaccharide from barley. It can explain why the effect on prolactin can also be induced by non-alcoholic beer, most likely to enhance

the antioxidant capacity of breastmilk and decrease oxidative damage in breastfeeding mothers [19].

5. Conclusion

In conclusion may be assert that the consumption of alcoholic beverages with a 5% ethanol content in both distilled and fermented forms during lactation caused alterations in the chemical composition of the breast milk in rats, with an increase in lactose caused by beer and an increase in potassium caused by the distilled beverage.

These findings suggest that the use of alcoholic beverages, even those with low ethanol content, during lactation can cause serious alterations in the metabolism of mothers, which are reflected in the composition of breast milk, possibly compromising the nutrition and normal development of newborns.

Acknowledgements

The authors thank the Brazilian agency FACEPE (Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco) for financial support.

List of Abbreviations

CG - Control Group
 AG - Alcohol Group
 PFAG - Peer-Fed Alcohol Group
 BG - Beer Group
 PFBG - Peer-Fed Beer Group

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