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Anxiety behavior is reduced, and physical growth is improved in the progeny of rat dams that consumed lipids from goat milk: An elevated plus maze analysis

Juliana K.B. Soares[a], Ana P.R. de Melo[a], Maria C. Medeiros[a], Rita C.R.E. Queiroga[b], Marcos A.D. Bomfim[c], Elaine C.A. Santiago[a], Rubem C.A. Guedes[a,*]

[a] Laboratório de Fisiologia da Nutrição Naíde Teodósio, Dept. Nutrição, Universidade Federal de Pernambuco, Recife, PE 50670-901, Brazil
[b] Departamento de Nutrição, Universidade Federal da Paraiba, Brazil
[c] Empresa Brasileira de Pesquisa Agropecuaria, EMBRAPA Caprinos, Sobral, CE, Brazil

HIGHLIGHTS

- We treated pregnant and/or lactating rat dams with a goat milk fat diet (GMF).
- We explored brain effects of GMF in the dams' progeny.
- We measured physical growth changes and behavior effects.
- We described growth improvement and anxiety reduction in GMF treated rats.
- We suggest that conjugated linoleic acid from GMF is involved in those effects.

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ABSTRACT

The goat milk contains conjugated linoleic acid (CLA), which can influence physical growth and brain development. This study investigated the impact of a diet containing goat milk fat (GMF) on physical parameters of gestating (G) and/or lactating (L) rat dams, and their progeny's physical growth, and anxiety behavior. In the dams, body weight was evaluated during gestation and lactation. Maternal physical parameters, thoracic and abdominal circumference and liver weight were measured at weaning. In the progeny, indicators of somatic development, and consolidation of reflex responses (palm grasp, righting, free-fall righting, vibrissa placing, auditory startle response, negative geotaxis and cliff avoidance) were determined. Anxiety behavior was tested on the elevated plus maze (EPM). Compared to the controls, GMF-pups presented higher body weight and tail length at days 18 and 21 (groups G + L and L). In the L-group, cliff avoidance and free-fall righting responses were respectively delayed, and accelerated. Fur appearance was anticipated in G + L pups. On postnatal day 35, the EPM responses of the G group indicated less anxiety than in the controls. Data show developmental and behavioral modifications in the progeny of dams fed the GMF-rich diet consumed during gestation and lactation, suggesting the involvement of CLA in such effects.

1. Introduction

Linolenic acid and linoleic acid are essential fatty acids that cannot be synthesized by the mammalian organism, and therefore have to be obtained from the diet. They can prevent the development of dysfunctions of the central nervous system and growth retardation [8]. During the last phase of fetal life and the suckling period, the brain undergoes a rapid growth [16]. During this phase, essential nutrients such as the essential fatty acids are considered as very important [24]. In the intrauterine life, the placenta is responsible for supplying the lipids to the fetus, and in the lactation period maternal milk is necessary for supplying the demand of these nutrients to the newborns [13]. Milk fat is the source of complex lipids, which are present in milk-derived bioactive products and may exert long-term effects on neural development and function [33]. Goat milk plays an important role in human nutrition [27]. It contains the conjugated linoleic acid (CLA), which comprise a mixture of geometric and positional isomers with double bonds varying at the

Abbreviations: CLA, conjugated linoleic acid; EPM, elevated plus maze; GMF, goat milk fat; C, control; G, gestation; L, lactation; G + L, gestation + lactation.

* Corresponding author at: Departamento de Nutrição, Universidade Federal de Pernambuco, BR-50670-901, Recife, PE, Brazil. Tel.: +55 81 21268936; fax: +55 81 21268473.
E-mail addresses: guedes.rca@gmail.com, rguedes@ufpe.br (R.C.A. Guedes).

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carbon chain of the linoleic acid molecule [19]. CLAs are formed by biohydrogenation in the rumen of ruminants by the action of rumen bacteria [19]. The goat milk is a good source of CLA. It is an alternative for infants and adults sensitive or allergic to cow milk [5,31]. Some reports established that CLA can have positive effects on the cardiovascular system [20] and on growth in rats [6]. CLA also may cross the blood-brain barrier [9], having anti-angiogenic action [15].

Dietary lipids also appear to influence the susceptibility to depression and anxiety [10]. The elevated plus maze (EPM) is considered a valid model to evaluate anxiety in animals, allowing the investigation of behavioral and neuropharmacological parameters [21,23]. Several factors such as gender and age [14], and nutritional status, including lipids [17] may influence the anxiety-like behavioral responses in the EPM.

Despite a growing literature describing therapeutic effects of CLA in disorders of peripheral organic systems, little is known about the neurobehavioral effects of this fatty acid, either ingested as such, or added to the food.

The hypothesis tested in this study is that a CLA-rich maternal diet, containing goat milk fat (GMF) as the only lipid source, when consumed during pregnancy and/or lactation positively influences physical maturation and the behavioral development of the pups. Thus, this study aims to investigate the impact of a CLA-rich maternal diet on reflex maturation, physical parameters and anxiety-like behavioral EPM responses in their offspring.

2. Materials and methods

Female Wistar rats aging 120–150 days and weighing 250 ± 50 g were obtained from our institutional vivarium and mated to obtain the progenies employed in this study. The animals were kept in standard conditions of temperature (22 ± 1 °C), 12–12 h light-dark cycle (light phase beginning at 6 a.m.), and relative humidity of 65%, with free access to food and water. Each litter was formed by four male- and two female pups. For the analyses performed in this work, only the male pups were used. The experimental procedures were approved by the Ethics Committee for Animal Research of our University, which complies with the recommendations from the National Institute of Health (Bethesda, USA).

The control (C)- and the goat milk fat (GMF)-diets were formulated based on recommendations of the American Institute of Nutrition (AIN-93G) [22]. To obtain the goat milk, we followed the general procedures described by Stanciuc et al. [30]. The animals were milked in the morning. Milk was stored at 4 °C in sterilized bottles until being centrifuged at 15,000 × g for 5 min to obtain the GMF, which was stored under refrigeration and used for manufacturing the diet by the company Rhoster (São Paulo/Brazil). The GMF was added in the proportion of 7 g per 100 g of diet. Both diets contained 20% proteins, 63% carbohydrates, 7% fat, 3.5% salt mixture, 1% vitamin mixture and 0.2% choline. The fat of the C diet was soybean oil. The physicochemical analysis of the diets confirmed the proportions of the ingredients, including GMF, which indicates that no other “contaminating” components were extracted together with GMF. Therefore, both diets differed in lipid quality, but not in quantity. The experimental diet contained 1.2% of CLA, whereas in the standard diet no CLA was detected (Table 1). In our previous study [29] we detected the presence of two isomers of CLA, namely c9t11 and t10c12 in the brains of the GMF diet-fed groups, but not in the control rats.

The pups from the GMF-treated dams were divided in three groups, depending on the period of the maternal GMF consumption: gestation, lactation and gestation plus lactation (respectively groups G, L and G + L). They were compared to the control group (C), suckled by dams receiving the control diet.

Table 1
Fatty acid composition of the diets.

	Control diet	GMF diet
C4:0	–	0.95
C6:0	–	0.96
C8:0	–	0.99
C10:0	–	3.60
C12:0	–	2.10
C14:0	0.42	6.40
C15:0	–	0.63
C16:0	17.10	24.26
C16:1	–	0.34
C17:0	0.34	0.53
C18:0	5.93	20.94
C18:1(n9)	28.53	28.79
C18:2(n6)	42.56	5.97
C18:2(n6)CLA	–	1.20
C18:3(n3)	1.89	0.63
C20:1	2.18	–
C22:0	0.53	–
ND	0.52	1.71

ND, not determined. Values are means.
GMF, goat milk fat.

The maternal body weights were determined on the gestation days 1, 7, 14 and 21. During the lactation period, the maternal weights were measured 24 h after delivery and subsequently at 7, 14 and 21 days. The newborns were weighed daily, after examining for reflex and somatic maturation (see below).

After weaning, the dams were weighed and anesthetized using ketamine hydrochloride and xylazine hydrochloride (1 ml/kg) for analysis of the following physical parameters: Body mass index (weight (g)/length^2 (cm^2)), thoracic- and abdominal circumferences (cm) and the ratio between abdominal and thoracic circumferences, as described by Novelli et al. [18]. At the end of these measurements, the dams were sacrificed by an overdose of anesthetic and their livers and abdominal fat were removed and weighed.

The consolidation of reflex responses was investigated daily (between 11 a.m. and 1 p.m.) as described elsewhere [28]. The day of consolidation was considered as the first of a series of three consecutive days in which the expected response appeared fully developed. The following reflex responses were studied: disappearance of Palmar Grasp; appearance of Righting, Vibrissa Placing, Cliff Avoidance, Negative Geotaxis, Free-fall Righting and Auditory Startle Response. For each response, the maximum observation time was set on 10 s.

The following indicators of somatic maturation were daily examined: ear unfolding, fur appearance, eruption of the inferior and superior incisor teeth, eye opening and tail length.

The EPM apparatus consisted of two open arms (50 cm × 10 cm) opposite to one another and crossed at right angles by two closed arms (50 cm × 10 cm × 40 cm), with a central square of 10 cm × 10 cm. The apparatus was elevated to the height of 50 cm as described by Pellow et al. [21]. It was located in a sound-attenuated room, which was illuminated by two 60 W red lamps installed on the roof of the room to a height of 2.5 m above the maze. At the beginning of the test session, the 35 day old animal was placed in the central square of the EPM with the head oriented to one of the closed arms. The following behavioral categories were analyzed: total time spent in the open arms; total time spent in the closed arms; total time spent in the central square; number of head dipping (when the rat looked under the open arms of the apparatus). After testing one animal, the maze was cleaned with a 10% alcohol solution and wiped dry, before the next animal was tested. Animals that fell from the EPM were returned to their home cage and excluded from subsequent analyses. The animals' behavioral reactions were recorded using a camcorder connected