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Review Article

Goat Milk Fatty Acids on Brain Growth and Functions: A Systematic Review

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Abstract

A systematic review was conducted to elucidate the role of goat milk fatty acids on neural development and functions. The search was systematically done on six databases; SCOPUS, EMBASE, Cumulative Index to Nursing and Allied Health Literature (CINAHL), OVID, Proquest Education Journal and PUBMED. The review was conducted until June 2018. The search focused on any studies related to the neural developmental effect of goat milk in human and animal brain. The search strategies used keywords related to “goat”, “milk” and “brain”. There was no restriction on study designs, publication date or language. A total of 3185 abstracts were initially identified. After reviewing the titles, abstracts and full papers using pre-determined inclusion and exclusion criteria, no human-related study was found. However, four studies showed positive effects on brain growth and development and reflex maturation in animals. Therefore, more research should be done to elucidate the relationship between goat milk and brain physiology especially in human.

Keywords: Brain; Goat Milk; Linoleic Acid; Lipid; Systematic Review

Abbreviations

CLA: Conjugated Linoleic Acid; CNS: Central Nervous System; CSD: Cortical Spreading Depression; DGW: Dried Goat Whey; DNA: Deoxyribonucleic Acid; EFA: Essential Fatty Acids; EPM: Elevated Plus Maze; NEU: Neuraminidase; ORT: Object Recognition Test; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; PUFA: Polyunsaturated Fatty Acid

Introduction

The goat milk is the milk of choice by those who are allergic to cow and sheep milk [1]. A study showed that allergic reactions towards goat milk were not as severe as cow milk [2]. Studies conducted by researchers stated that weight gain, height, and blood serum contents of calcium, thiamin, vitamin A, riboflavin and hemoglobin in human and rats who had consumed goat milk surpassed those who consumed cow milk [4,5]. Biochemical composition of goat milk may varies according to the breed, type of food, lactating state and environmental conditions [6]. Due to these factors, the quantity and the quality of goat milk fat content such as omega-6 and omega-3 also varies [7].

Goat milk fat is one of the most complex natural fats comprising approximately 400-500 fatty acids. Omega-3 and omega-6 fatty acids are important for brain development [8]. Essential fatty acids (EFA) is one of the subsets of fatty acids involved in brain development especially in neonates, which are incapable of synthesizing their own EFA [9]. During pregnancy and lactation, polyunsaturated fatty acids are crucial for neonatal development where the supply of these fatty acids is derived from maternal nutrition [10-13].

Polyunsaturated fatty acids are initially catalysed by enzymes initially into two precursors: linoleic acid (18:2) and alpha-linoleic acid (18:3) [14-16]. Linoleic acid consists of a few geometrical isomers which are generally designated as conjugated linoleic acid (CLA) [17]. CLA is found in goat milk. Linoleic acid from the omega-6 group and linolenic acid from the omega-3 group are important for the development of the nervous system especially in the final trimester of intrauterine life. During this stage of pregnancy, brain development is accelerated [18].

The quality and the amount of fatty acids are important for the development of the neonatal nervous system. Malnutrition impairs brain growth and the development by decreasing replication of the cell cycle, reducing brain total deoxyribonucleic acid (DNA), and restricting dendritic arborization [19]. Thus, these activities will reduce neuronal connectivity. Deprivation of the essential nutrients in the neonate and toddler will affect the development of the cerebellum, particularly on its synaptic connectivity [20,21]. Furthermore, the EFA will mainly affect brain structural composition and myelin sheaths [10]. These include alterations in the electroencephalographic activity, motor and cognitive development, visual and auditory evoked responses and social abilities [19].

Goat milk has been highlighted as one of the high quality raw materials for food manufacturing [22,23]. Most of the studies conducted were mainly on neurodegenerative diseases and microbiological effects of goat milk. However, the ability of goat milk to improve brain development and functions is still unknown. Based on many effects of goat milk fatty acid on neural development and functions, it is important to systematically review the present literature on the benefits of goat milk beneficial to brain development. This helps in explaining goat milk effects on brain development and function.

Methods

A comprehensive and updated database was used to catalogue the literature on goat milk and its influence on brain development and function. The evidence database was assembled according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for systematic reviews (http://www.prisma-statement.org/). The method was adopted from Cochrane Reviewers' Handbook.

Search methods for identification of studies

Computer search

A broad search for the literature was performed by incorporating both electronic and manual components. The electronic search was accomplished using SCOPUS, EMBASE, Cumulative Index to Nursing and Allied Health Literature (CINAHL), OVID, Proquest Education Journal and PUBMED, which were conducted until June 2018. Based on the databases, relevant papers via the main keywords: goat, milk and brain were identified. Further search terms are presented below. The study was accomplished through the screening steps by referring to the inclusion and exclusion criteria.

The search terms strategies include all terms related to main keywords as follows:

1. Goat*: capra*; caprine*; hircus*; OR hircine*;
2. Milk*: dairy*; butter*; cream*; yogurt*; cheese*; OR whey*;
3. Brain*: nerv*; cereb*; cortex*; cortical*; OR neuro*;

An asterisk after a term means that all terms that begin with that root were included in the search.

Step 1

All the identified and relevant abstracts were compiled in an individual library in Endnote (X7.0.1) software. All the duplicated data identified and removed from the library. Abstracts were reviewed based on the following inclusion criteria: 1) human and animal studies, 2) sources from goat milk and its derivative products and 3) studies related to brain and central nervous system (CNS). If the criteria were not met because of inadequate information, the abstract was set aside for further evaluation.

Step 2

The abstracts were reviewed independently by three investigators and were selected based on their consensus according to the same criteria used in Step 1. If the consensus was not reached, the abstract was set aside for further evaluation.

Step 3

Full-text articles of abstracts selected in Step 2 were retrieved and reviewed. Studies were included if they met the following criteria: 1) used transgenic mice, 2) involved goat milk's fatty acids and 3) showed measurements of a central nervous system and physical activity. The exclusion criteria were 1) studies that did not discuss the effectiveness of the goat milk relevant to the brain activity, 2) studies that involved drugs as intervention and 3) studies that involved goat gene as milk promoter.

Review of reference lists

The reference lists of all articles selected in Step 3 were reviewed, and the full texts of the potentially related studies were examined.

Data Extraction

For data extraction, the screened studies had to report the outcomes based on the following conditions: *In vitro* study, related to goat products consumption and neural developmental effect. There were no restrictions in the study design and publication date. The adapted data extraction form (Appendix A) include information about the authors, selection procedure, analyses, animals' characteristics, type of intervention(s), characteristics of methodological quality, and results.

Heading	Subheading	Description	For completion by reviewer(s)			
Bibliographic details	Title					
	Authors					
	Publication date					
	Study aims	What were the study's aims and purpose?				
Ethical standards		Was ethical committee approval obtained? Was informed consent obtained? Does the study address ethical issues adequately? Has confidentiality been maintained?	Ethical approval	Yes	No	Unclear
			Informed consent	Yes	No	Unclear
			Ethical issues addressed	Yes	No	Unclear
			Confidentiality maintained	Yes	No	Unclear
Context	Aims	Are the aims and purpose of the study clearly stated?		Yes	No	Unclear
Setting	Area and care setting	What is the geographical and care setting for the study?				
	Rationale	What is the rationale and appropriateness for this choice?				
	Detail	Is there sufficient detail about setting?		Yes	No	Unclear
	Timing	Over what period did the data collection take place?				
Sample	Inclusion criteria	Who was included in the study?				
	Exclusion criteria	Who was excluded in the study?				
	Selection	How was the sample selected? Were there any factors that influenced how the sample was selected (e.g. access, timescale issues)?				
	Size	What is the size of the sample and groups comprising the study?				
	Appropriateness	Is the sample appropriate in terms of its ability to meet the aims of the study, the depth of data that it is enables to be collected, and its breadth?		Yes	No	Unclear
Data collection	Methods	What data collection methods were used?				
		Was the data adequately described and rigorously conducted?		Yes	No	Unclear
	Fieldwork	Is the process of fieldwork adequately described?		Yes	No	Unclear
	Data analysis	How are the data analyzed? How adequate is the description of the data analysis? Is adequate evidence provided to support the analysis (e.g. use of original data, iterative analysis, efforts to establish validity and reliability)? Is the study set in context in terms of findings and relevant theory?				
	Researcher's potential bias	Are the researcher's /researchers' own position, assumptions and possible biases outlined? Indicate how they could affect the study in terms of analysis and interpretation of the data				
	Reflexivity	Are the findings substantiated by the data and has consideration been given to any limitations of the methods or data that may have affected the results?		Yes	No	Unclear
Outcomes	Outcomes	What outcome measures were adopted? What was the impact of the study for (a) service users (b) careers (c) practitioners (d) organization responsible for service				
Findings	Themes					
	Conclusion					
	Opinions	What this person argues?				

Appendix A: Data Extraction form (Supplementary data).

Risk assessment

All the studies were screened to confirm that goat milk was given as supplementation as included in the methodology. Once the studies were confirmed to show the effectiveness of goat milk consumption on the central nervous system and physical activity, the studies were categorised as potential studies for further evaluation.

Summary measurement

This study was conducted qualitatively to synthesise the effects of goat milk on neural development. Meta-analysis was not attempted due to the exploratory design of the study.

Results

A flow diagram in Figure 1 has outlined the systematic review process of the research result selection and the reasons of exclusion. The search strategies initially identified 3185 abstracts from six databases. After duplicates abstracts were removed, there are 2702 abstracts remained. By screening the titles and abstracts, seven articles were selected for full-text review where among them, no human-study was retrieved and four of them are animal-related studies which focusing on physical and brain development and reflex maturation. One of the studies used goat milk gene promoter, hence it was excluded.

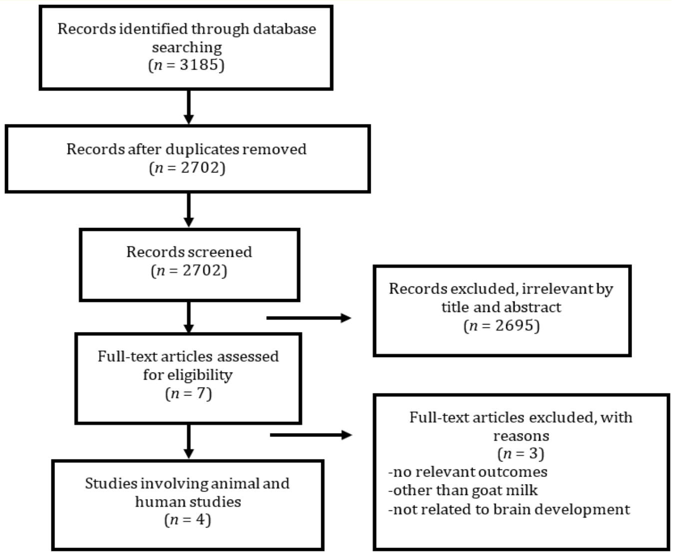

Figure 1: Flow diagram of study selection. Note: n = number of publications.

Study characteristics

Three out of four studies that fit the criteria were conducted by the same group of researchers between the year of 2012 to 2014 [9,24,25]. The studies discussed the effect of conjugated linoleic acid of goat milk on the brain. Two of the studies compared goat milk fat (experimental; n = 83) and soybean oil (control; n = 27), while other studies compared dietary lipids from goat milk (n = 24), soybean (n = 18), and coconut oil (n = 12). Another similar study was conducted by Medeiros., *et al.* [26] where the study was conducted using dried goat whey. Table 1 summarises the findings of all four studies.

Risk of bias across studies

All the studies were conducted in the same laboratory. The rat diet which exclude goat milk was not discussed thoroughly in the methodology. Therefore, it cannot be concluded that the goat milk supplementation was beneficial to the brain development.

Study intervention

According to Soares., *et al.* [9,24,25], female Wistar rats that had been mated to obtain a few progenies for further analysis were used in the three studies. Each diet differs in the composition of the saturated-to-polyunsaturated fatty acid ratio [9,24,25]. The experimental group received a diet containing 7% fat from goat milk. In this study, the experimental rats were divided into three groups. The first group (n = 27) of rats were fed with goat milk during the gestation phase. The second group (n = 28) of rats were fed with goat milk during the lactation phase, and the third group (n = 28) of rats were fed with goat milk from gestation phase up to 35 to 45 days of lactation phase.

Medeiros *et al.* [26] tested only male pups in their study. This study focused on the effects of sialic acid (SA) content in goat whey on memory and cortical spreading depression (CSD). In this study, the experimental pups were divided into two major groups; litters with nine (L_9) (n = 51) and fifteen (L_{15}) (n = 52) pups, respectively. There are five subgroups in each group; dried goat whey (DGW), SA20 (20 mg/kg/day), SA100 (100 mg/kg/day), saline solution and naive groups. The pups administered DGW and SA via gavage. No gavage was introduced to control groups; saline and naïve groups. The DGW received 17.45 g/kg/day dried goat whey (DGW), which equivalent to 20 mg of SA. The whole experiment required samples size of 51 L_9 and 51 L_{15}.

Author and Year	Milk Composition	Animals	Experimental design	Parameters	Result
Soares., *et al.* 2012	7% fat from goat milk	Female Wistar rats Aged 120-150 days of life Weighing 300 ± 50 g were mated	1 control group (soybean oil); n = 27 3 experimental groups (goat milk); n = 83	Body weight Cortical spreading depression (CSD) recording	Body weight increased in *lactation* and *gestation+lactation* groups (p < 0.05) Increase CSD phenomenon (p < 0.05)
Soares., *et al.* 2013	7% fat from goat milk	Female Wistar rats Aged 120-150 days of life Weighing 250 ± 50 g were mated	1 control group (soybean oil); n = 27 3 experimental groups (goat milk); n = 83	Body weight Anxiety behaviour Reflex responses	Pups in all groups showed higher body weight *Gestation* group indicated less anxiety than control group (p < 0.05) In the *lactation* group, cliff avoidance and free-fall righting responses were respectively delayed and accelerated (p < 0.05)
Soares., *et al.* 2014	7% fat from goat milk	Female Wistar rats Aged 120-150 days of life Weighing 250 ± 50 g were mated	Soybean oil group; n = 18 Coconut oil group; n = 12 Goat milk fat group; n = 24	Body weight Reflex responses	Lipids from goat milk increase body weight in all groups Acceleration of reflex maturation (p < 0.05)
Medeiros., *et al.* 2016		Male pups Wistar rats	20 mg DGW; n = 19 SA20; n = 21 SA100; n = 20 Nv; n = 21 Sal; n = 20	Body weight Recognition task (Spatial position, shape and novel object) Cortical spreading depression (CSD) recording	DGW, SA20 and SA100 showed increased in body weight (p < 0.05) Longer exploration time was presented in DGW group (p < 0.05) Increased CSD propagation velocities (p < 0.05)

Table 1: Summary of included studies (in chronological order).

Outcomes

All studies examined the effect of fat (conjugated linoleic acid) and sialic acid contained in goat milk and dried goat whey, respectively, based on cortical activity. The effects of diet on reflex maturation, memory performance and anxiety behaviour were also discussed [9,24-26].

Reflex response

The pup's reflexes ontogeny (maturation process) was investigated based on palm grasp, righting, free-fall righting, vibrissae placing, auditory startle response, negative geotaxis and cliff avoidance. Goat milk supplementation improved reflexes response and somatic maturation in pups. It was reported that cliff avoidance and free-fall righting responses were respectively delayed and accelerated in all three experimental groups especially in the lactation group [9,24].

Cortical activity

The study by Soares., *et al*. [24] focused on the influence of the dietary lipids on neuronal excitability. The investigation was conducted using the electrophysiological activity known as cortical spreading depression (CSD). In both gestation and combined gestation-lactation groups of goat milk fat consumption, there is a significantly higher in number of multiple episodes occurred in these groups. Moreover, the propagation of CSD velocities in both lactation and combined gestation-lactation group were higher than the control and gestation groups. This is in line with a study where the experimental groups show a higher propagation of CSD velocities compared to the control groups [26]. Leo [27] had described that CSD is a fully reversible and excitability-related neural response. It is a slow propagation wave of depression of spontaneous neuronal activity from electrical, mechanical or chemical stimulation of one point on brain tissue, from which it spreads concentrically to remote cortical regions.

Anxiety behavior

Elevated plus maze (EPM) was used as a model for testing the anxiety behaviour of pups [9]. This test was used to characterise the exploratory and anxiolytic effects based on the recorded time spent in the maze. The total time spent in the open and closed arms as well as in the central square were recorded and the number of head dipping was counted. The anxiety level was reduced in dams and pups supplemented with goat milk and the behavioural effect was minimised. The duration of time that the pups remain stationary at the open arm of the EPM was increased associated with the increased in the number of head dipping. However, the pups tend to spend less time at the centre of the maze.

Memory and behavioural test

A specific test named object recognition test (ORT) is a behavioural assay that is commonly used in order to investigate animal's learning and memory. This test helps to evaluate the efficacy of an animal to recall identical objects after a new object was switched with one of the objects in the apparatus. The rat will spend more time on the new object except for the rat with a memory deficit. Longer exploration time was taken in DGW groups.

Discussion

The systematic review was conducted to study the effect of goat milk on neural development and functions. The result revealed that three studies by the same author recorded the benefits of fatty acid in goat milk, which enhance brain development and functions [9,24,25]. The main results from all three studies showed an increase in body weight, and improvement in reflex response and brain development after a period of goat milk fat consumption. In addition, anxiety-like behaviour with an anxiolytic effect and cortical activity were also discussed. These experimental studies were conducted on rat dam and its progeny.

The fatty acids composition of both experimental and control diets revealed a different ratio between saturated and polyunsaturated fatty acids. The experimental diet of goat milk recorded CLA in fatty acids composition, whereas no CLA was detected in the standard diet [9,24,25]. Based on all three studies that have been conducted by Soares., *et al.* [9,24,25], chronologically from the year 2012 to 2014, CLA is crucial for the CNS development, especially during pregnancy and lactation period.

Another study revealed the benefit of sialic acid on pups memory after the consumption of dried goat whey [25]. Goat whey, which that rich is sialic acid is a by-product that was discarded after goat cheese manufacturing. The experimental groups showed a positive result in object recognition test (ORT). The rats took a longer time to explore the new object among old objects that were placed in the apparatus concluding that sialic acid is a good source for memory and brain excitability processes.

Reflex response

The consumption of goat milk fat shows an acceleration in reflex maturation compared to control group, such as soybean oil and/or coconut oil [24,25]. CLA content in goat milk lipid might be responsible for the change in the acceleration and the reflex maturation of the pups. These findings were consistent with the previous studies, where rats fed with diet supplementation showed an acceleration of reflexes [14]. These findings demonstrate that the changes in maternal dietary fatty acids may facilitate the reflex ontogeny in the offspring.

During the postnatal period, free-fall righting responses can only be observed once the central nervous system has been fully developed [28]. Besides, vestibulospinal, visual and somesthetic afferents are also directly related to posture correction [29]. There are a few developmental events that are able to generate such reflex responses. The events include neuronal and glial proliferation and the establishment of synaptic contacts [30], as well as myelination [18], which are directly related to dietary lipids. Goat milk fat diet consumption showed that pups are able to turn in mid-air to land on its four paws [23,24]. Therefore, during the gestation period, the accumulation of fatty acids in the brain is crucial for fetal development [10]. Hence, the accumulation of fatty acids is very important during the rapid development of nervous system which was seen in the ontogeny reflex [31].

Cortical activity

Leo [27] discovered that the initial phase of cortical spreading depression (CSD) was characterised by the depression of the spontaneous brain electrical activity and the electroencephalography depression spread slowly all over the brain cortical surface. The increased in CSD is associated with the pathophysiological events of ischemia, migraine and epilepsy in human [32,33]. By referring to the previous work published, the measurement of the physical parameters required an anaesthesia [25,26]. Therefore, at this stage, no changes were seen by the physical parameters in the experimental groups. In contrast, the CSD propagation in the experimental groups showed an activity in brain electrophysiological even though an absence of weight difference. Thus, this suggested that the CLA and sialic acid effects on body development were short lasting compared to CSD propagation, where long-lasting effects on brain electrophysiological were observed in pups growth.

The CLA would impair brain myelination, a development process in mammals to reach the maximal intensity during the lactation period. Modification in dietary lipids has been proven to affect on the myelination. A study reported that CSD propagations either accelerated or decelerated, were associated with demyelination and hypermyelination, respectively [34]. According to Borba., *et al*. [35] showed that the dam and progeny which received diet rich in saturated fatty acid caused an increased in myelination and decelerated the CSD. In contrast with Soares., *et al*. [24] and Medeiros *et al*. [26], an acceleration of CSD may be due to the diet used which probably impaired myelin synthesis in the progeny. It is a fact that CSD plays an important role in human neurological disorders like epilepsy [32]. An increase of epilepsy prevalence was noted among patients who have been diagnosed with multiple sclerosis [36] and demyelinating disease [37,38]. Moreover, the key enzyme that cleaves SA, known as neuraminidase (NEU), has been proven to have better anticonvulsant action [39]. This was in line with the study conducted by Isaeva., *et al*. [39,40]. Still, further experiment on brain myelin needs to be quantified to show the relationship of this hypothesis.

In the study by Soares., *et al*. [24], the *gestation* and the combined *gestation-lactation* groups of rats consuming fat from goat milk showed a significantly high multiple episodes of CSD. Normally, once potassium chloride (KCl) was stimulated, a single CSD episode will be elicited, but in this study, “multiple” CSD episodes occurred with a single potassium chloride (KCI) stimulation. As supported by Bourre [31], the brain that is susceptible to the CSD phenomenon could be observed from these “multiple” CSD episodes. This result showed that goat milk may increase CSD phenomenon, which is an adverse reaction of goat milk. However, the reason for the increased of CSD in their study may be due to the diet of goat milk that is deficient in both n-3 and n-6 fatty acid, which is important for an adequate brain development [15]. Moreover, the goat milk has high PUFA content, which protects the brain from damage by inducing robust dilatation of the cerebral vessels [41].

Anxiety behaviour

Anxiety disorder can interfere in the learning process by impairing attention and memory. Thus, to measure the anxiety level or to characterise an exploratory and anxiolytic effect, elevated plus maze apparatus has been used. The elevated plus maze (EPM) has been designed to accomplish the anxiety test conducted on animals where the main target was to measure the time taken for an animal to be in different position.

Pups that consumed goat milk fat exhibited an increase and decrease of the time spent in the open arm and centre part of the maze, respectively, which were significantly different as compared to the control group. Head dipping recorded was also increased recorded by the goat milk fat consumed group. According to Soares., *et al*. [9], goat milk fat consumption especially n-3 fatty acid is associated with the decreased of anxiety-like behaviour in the plus maze test. Once the anxiety becomes pathological, it will impair the attention and memory which then disturbed the learning processed [42]. Similarly, this study discusses the work presented by Sharma., *et al*. [43] and Sivanathan., *et al*. [44], where the anxiety behaviour was also reduced when the rat consumed hypercaloric diet. This reinforces the idea that the changes in anxiety behaviour in the progeny were mainly caused by the consumption of goat milk fat which consequently affects the pups’ nervous system. This is not due to the dams’ nutritional deficiency. It is suggested that the CLA has been postulated to contribute a positive effect on the anxiety behaviour (less anxiety) as compared to the control group.

Memory and behavioural test

Memory and learning impairment may interfere with the spatial cognitive ability. In order to measure the spatial cognitive ability, object recognition test (ORT) was applied using a circular arena apparatus with a diameter of 89 cm. This test was conducted on animals, where the main objective was to measure the time taken for an animal to recognise the novel object among other objects that had been placed in the same apparatus.

Pups that consumed dried goat whey (DGW) required more time during the recognition of the new object showing a significant difference as compared to the control group. Once memory impairment becomes pathological, it will impair the behavioural and electrophysiological actions. According to Medeiros., *et al.* [26], DGW contains sialic acid (SA) received by gavage, associated with the improvement of object recognition memory of an animal. Similarly, this study discusses the work presented by Limon., *et al.* [45] and Wang., *et al.* [46], where memory and learning test were also demonstrated to perform better when the pups consumed with sialic acid. However, the effect was not caused only by sialic acid (SA), but other nutrients such as taurine [47,48] contained in DGW were necessary to support the memory and learning ability. This reinforces the idea that the changes in memory effects among the pups were mainly caused by the gavage of DGW, resulting in the direct action on the pups' memory and learning ability. It is suggested that the SA has been postulated to improve the spatial cognitive ability when compared to the control group.

The present study has several limitations. Although the search strategies are not limited to human and animal studies, there was lack of evidence on the effect of goat milk on brain activity. This may also due to the collected data from unpublished papers that were not indexed in the databases and those that are unable to be retrieved. However, the limited findings suggest a relationship between the goat milk fat consumption by the maternal rats with the physical development, cortical activity, and anxiety behaviour in the progeny. Due to the small number of studies on goat milk consumption related to brain activity, future research is required to provide the evidence that goat milk consumption could affect brain activity in the rat. The data currently gathered was limited because the work was mainly conducted by the same authors. The papers did not discuss clearly the methodology of the experimental studies, especially regarding the rat's diet. Thus, the information whether the dams and the progeny were given any other food was not clearly stated. To date, the recorded goat milk effects were all positive with respect to its body development (improvement in physical growth), the central nervous system (reduction in anxiety behaviour), and cortical activity (susceptible to the CSD phenomenon).

Conclusion

In conclusion, there is a need to provide more evidence on the effects of goat milk consumption on brain activity among human and animal. More research should be conducted to strengthen the relationship between goat milk consumption and brain activity.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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