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Short Communication

Potential of soybean-velvet bean combination tempe in improving cognitive function

Made Astawan[1*], Aprilia F. Damayanti[1], Tutik Wresdiyati[2], Diana N. Afifah[3] and Irma S. Rahmawati[4]

[1]Department of Food Science and Technology, Faculty of Agricultural Engineering and Technology, Institut Pertanian Bogor, Bogor, Indonesia; [2]School of Veterinary Medicine and Biomedicine, Institut Pertanian Bogor, Bogor, Indonesia; [3]Department of Nutrition Science, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia; [4]Department of Nutrition, Faculty of Medicine Science, Universitas Brawijaya, Malang, Indonesia

*Corresponding author: astawan@apps.ipb.ac.id

Abstract

Velvet bean is a native Indonesian legume containing L-dopa, yet it remains underutilized. The aim of this study was to analyze the effects of different types of tempe (soybean, velvet bean, and their combination) on cognitive function, brain histology, dopamine levels, and serum β-amyloid in rats, as well as to identify the parameters most influencing cognitive function, including brain mass and volume, hippocampal neuron count, and dopamine and β-amyloid levels. An experimental study was conducted using a completely randomized design with one factor: the protein source of diet. Five rat groups were included based on protein sources: non-protein, casein control, soybean tempe flour, velvet bean tempe flour, and a combination of soybean and velvet bean tempe flour. This study examined cognitive performance by measuring maze completion time, while brain mass and volume, hippocampus histology for neuron cell counts in the dentate gyrus section, cornu ammonis 1 (CA1), and cornu ammonis 3 (CA3), brain dopamine and β-amyloid serum levels were measured after eight weeks of intervention. Our data indicated that rats deprived of protein had significantly slower maze completion times ($p<0.001$), underscoring the importance of protein in cognitive processes. Rats treated with non-protein rations had significantly lighter brain masses ($p<0.001$) than other treatments. Histological analysis of the hippocampus showed that the three types of tempe rations helped maintain the number and density of neuron cells in the dentate gyrus and CA3 of the hippocampus. Additionally, the protein in the ration could increase dopamine levels and suppress serum β-amyloid levels. There was a strong correlation between brain volume and neuron cell density in the dentate gyrus section of the hippocampus with cognitive function. These results highlight the promising role of combination tempe in increasing cognitive function, brain mass and volume, dopamine levels, and suppressing serum β-amyloid.

Keywords: Parkinson's disease, tempe, soybean, velvet bean, cognitive function

Introduction

Velvet bean, a native Indonesian agricultural product, remains relatively unknown and underutilized. Its use is primarily confined to serving as a raw material for making tempe. However, velvet bean tempe is less popular than soybean tempe due to its firmer texture, less appealing appearance, and less desirable taste. Despite this, velvet beans are nutritionally rich, comprising 31.79% protein, 4.52% lipids, 3.38% minerals, 54.75% carbohydrates, and 5.56% water [1]. Furthermore, the seeds and shells of velvet beans contain the bioactive compound L-

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dopa, with concentrations of 7.56% and 3.89%, respectively [2]. L-dopa is a precursor of dopamine and is currently synthesized (levodopa) for use as a treatment in individuals with Parkinson's disease [3], who often experience cognitive impairment. However, synthetic L-dopa can cause side effects and require progressively increasing doses [4].

The natural L-dopa found in velvet beans offers promising potential for managing neurodegenerative diseases that impair cognitive function. A trial involving the administration of velvet bean powder as a therapy for individuals with Parkinson's has been conducted, but participants faced challenges in tolerability, particularly due to gastrointestinal side effects, such as nausea [5]. To address these issues, technological advancements such as the fermentation process used in tempe production offer a promising solution. By combining soybeans with velvet beans, it is expected that the physical and nutritional qualities of the resulting tempe can be improved, making it a more viable option for consumption [6]. The aim of this study was to analyze the impact of different types of tempe (soybean, velvet bean, and their combination) on cognitive function, brain histology, brain dopamine levels, and serum β-amyloid in experimental rats. In addition, this study aimed to identify the parameters (brain mass and volume, the number of hippocampal neuron cells in the dentate gyrus section, cornu ammonis 1 (CA1) and cornu ammonis 3 (CA3), brain dopamine levels, and serum β-amyloid levels) that most influence cognitive function.

Methods

Study design and setting

An experimental study was conducted using a completely randomized design (CRD) with a single factor, the source of protein in the diet. There were five groups included in this study based on the protein sources in the ration: (a) a group of rats without protein content in the ration (non-protein); (b) a group of rats given casein (casein control); (c) a group of rats given a soybean tempe flour (soybean tempe); (d) a group of rats given velvet bean tempe flour (velvet bean tempe) and; (e) a group of rats given a combination of soybean and velvet bean tempe flour (soybean-velvet bean combination tempe). Each group consisted of five rats. During the study period, each rat was placed in an individual cage and was given a ration in the form of flour formulated with a variety of protein sources based on the groups (casein, soybean tempe, velvet bean tempe, or a combination of soybean and velvet bean tempe), as well as corn oil, mineral and vitamin premixes, carboxymethyl cellulose (CMC), water, and corn starch. The ration and drinking water were served *ad libitum* to each group of rats. This study was conducted at the Seafast Center Laboratory for Experimental Animals, Intitut Pertanian Bogor, Bogor, Indonesia. The research design was registered in the preclinical trials.eu with assigned protocol identification number PCTE0000536 (https://preclinicaltrials.eu/database/view-protocol/536). Histological analysis was conducted at the Histology Laboratory of the School of Veterinary Medicine and Biomedicine, Institut Pertanian Bogor, Bogor, Indonesia. The brain dopamine levels and serum β-amyloid levels were measured using ELISA kits at the Primate Animal Study Center, Institut Pertanian Bogor, Bogor, Indonesia.

Sampling strategy and inclusion criteria

The number of rats used in the study was calculated using the Federer formula and resulted in a total of five rats for each treatment group as a replication. Therefore, the total number of rats used in this study was 25. The inclusion criteria were male white rats of the Sprague-Dawley strain aged four weeks with a weight of 80–90 grams. Unhealthy rats and those weighing less than 80 grams were excluded.

Randomization and animal acclimatization

The acclimatization was conducted for three days to accustom the rats to consuming rations of flour. The rats were given casein rations and drinking water *ad libitum* during acclimatization. Then, their body weight was recorded for randomization and grouping. Rats that met the inclusion criteria were then randomized into five treatment groups. The variation in body weight of rats within each group was no more than 10 grams, while the average variation between treatment groups was no more than 5 grams.

Intervention

The ration intervention was conducted for eight weeks according to the treatment groups (non-protein group, casein/control group, soybean tempe group, velvet bean tempe group, and velvet bean-soybean combination tempe group). To establish a baseline, casein, a protein with a 100% digestibility rate, was employed as the control group. A non-protein group was included to ascertain the cognitive performance of rats deprived of dietary protein. The three distinct tempe varieties were incorporated to investigate their potential influence on cognitive function. Rations were provided *ad libitum*.

Production of tempe, tempe flour and ration formulation

The production of tempe involved creating tempe and tempe flour, which were used as rations for experimental rats. Three types of tempe were prepared: 100% soybean tempe, 100% velvet bean tempe, and a combination tempe made from 69% soybeans and 31% velvet beans. The tempe was produced at Rumah Tempe Indonesia (RTI) in Bogor, Indonesia.

The preparation process for velvet bean tempe began with soaking the seeds for 48 h at 30°C, followed by boiling them at 100°C for 95 min. The seeds were then dehulled, resoaked for 48 hours at 30°C, and washed to remove mucus. A second boiling at 100°C for 15 min was done, after which the seeds were drained. Yeast (2g/kg velvet bean) was added, and the mixture was packed in polyethylene plastic and fermented at 30°C for 48 h. For the soybean-velvet bean combination tempe, soybeans and velvet beans were mixed in a 69:31 ratio, packaged, and fermented under the same conditions [7].

Due to the smaller size of the beans and different anti-nutritional content, soybeans' preparation stage was shorter compared to the preparation stage of velvet beans. The washed soybeans were soaked for 2 hours at 30°C, then boiled for 15 min at 100°C. The soybean skins were crushed using a dehuller machine and then resoaked for 12 h at 30°C. The soybeans were separated from their skin and washed, then boiled again for 15 min at 100°C. There is no difference in the fermentation process between soybeans, velvet beans, or combination beans in tempe production [8].

Tempe flour was produced following the seven stages established in a previous study [9]. Proximate analysis was performed on the flour and used to prepare the ration formula. The proximate analysis included water, ash, fat, protein, and carbohydrate content, and all analytical procedures followed the 2012 guidelines of Association of Official Analytical Chemists (AOAC) [10]. The design of the rat ration adhered to the AOAC standard [10]. The experimental diets were formulated to be isoproteins, containing 10% crude protein, 8% crude fat, 5% moisture, 1% crude fiber, 1% vitamins, 5% minerals, and 70% carbohydrates. The protein source varied by treatment group, while corn oil, carboxymethyl cellulose (CMC), and cornstarch were consistently used as sources of fat, fiber, and carbohydrates, respectively.

Analysis of cognitive function using a maze test

This assessment was performed using the classic maze test during the seventh to eighth weeks of the feeding intervention. In the seventh week, the rats underwent an adaptation process to the maze environment. The animal subjects were placed in the maze and guided until they reached the finish line. This adaptation process was repeated three times on consecutive days. The following day, the animals' cognitive function was evaluated. Before the evaluation, the animals were fasted for six hours to induce hunger. The assessment was conducted by placing food at the finish point; then, the rats were given five minutes to complete the maze [11]. The testing procedure was replicated four times, with a one-day interval between each test. The time the rat reached the finish point was recorded as a cognitive function. The faster the rat reached the finish point, the better the rat could remember the maze, suggesting good cognitive function.

Brain mass and volume analysis

After eight weeks of treatment, the rats were terminated. Rats were anesthetized with an intraperitoneal injection of a ketamine (70 mg/kg body weight) and xylazine (10 mg/kg body weight) mixture. Once full anesthesia was confirmed, blood was collected intracardially for β-amyloid analysis, and the brains were removed [12]. The brains were placed in a 0.9% physiological NaCl solution, dried with tissue paper, and weighed. To measure brain volume, the volume change was assessed in a measuring cup containing physiological NaCl.

Histological analysis

Hippocampus brain tissue was fixed using Bouin's solution after which the tissue was embedded using the standard method and sectioned with a rotary microtome to a thickness of approximately 4 μm. The tissue sections were then stained with hematoxylin-eosin [13]. Neuron cells in the hippocampus were counted per field of view using a light microscope equipped with a camera at 400× magnification. The cell counts were analyzed with the ImageJ application (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, Maryland, United States).

Dopamine analysis

The rat brain tissue samples (±0.5 g) were prepared as homogenates at a tissue-to-phosphate-buffered saline (PBS) ratio of 1:9 (mass/volume). The homogenate was then centrifuged for 5 min at 5000×g and at a temperature between 2–8°C [14]. The supernatant was collected for dopamine level analysis using DA (Dopamine) ELISA Kit (Elabscience Biotechnology Co., Ltd., Wuhan, China) following the manufacturer's protocol. The absorbance was read using a microplate reader at a wavelength of 450 nm. Dopamine levels in the rat brain are presented in ng/100g units.

Serum β-amyloid analysis

Serum β-amyloid levels were analyzed using a Rat Aβ1-42 (Amyloid Beta 1-42) ELISA Kit (Elabscience Biotechnology Co., Ltd., Wuhan, China) following the manufacturer's protocol. Briefly, intracardial blood was collected in ethylenediaminetetraacetic acid (EDTA)-containing tubes and was centrifuged at 3000 rpm for 15 min to collect the serum. The serum preserved at -20°C before β-amyloid analysis. A 100 μL serum sample was added to each well, and the absorbable was read using a microplate reader at a wavelength of 450 nm. Serum β-amyloid levels in the rat are presented in pg/mL.

Statistical analysis

The data were analyzed by one-way ANOVA to compare among groups. If there was a significant difference in the measured parameters due to the treatment given, the analysis was continued using the Duncan multiple range test (DMRT) to determine which treatment had a significant difference at the 5% level. Pearson correlation analysis was conducted to determine the strength of each parameter's influence on the rats' cognitive function. Statistical analyses were performed using SPSS version 22 for Windows (SPSS Inc., Chicago, United States).

Results

Proximate composition and ration compositions

Based on the proximate analysis of the flour samples, presented in **Table 1**, the combined tempe exhibited characteristics that were not significantly different from soybean tempe in terms of moisture content, ash content, and protein content. The combined tempe had a moisture content that was not significantly different (p=0.159) from soybean tempe but was significantly higher (p<0.001) compared to casein, and lower (p<0.001) compared to velvet bean tempe. The combined tempe also showed protein (p=0.368) and ash content (p=0.721) that were not significantly different from soybean tempe, but both were significantly higher (p<0.001) compared to velvet bean tempe and lower (p<0.001) when compared to casein (**Table 1**).

In terms of fat content, the combined tempe had significantly lower fat levels compared to soybean tempe (p<0.001) (**Table 1**). However, its fat content was significantly higher (p<0.001) when compared to velvet bean tempe and casein. The crude fiber content of the combined tempe was not significantly different (p=0.608) from that of velvet bean tempe and was significantly higher compared to casein. However, when compared to the crude fiber content of soybean tempe, it was still significantly lower (p=0.006). Regarding carbohydrate content, the combined tempe had significantly higher (p<0.001) carbohydrate levels compared to soybean tempe and casein. However, when compared to velvet bean tempe, its carbohydrate content was still significantly lower (p<0.001) (**Table 1**).

Table 1. Proximate composition of casein and three types of tempe flour

Parameters	Sample flour				p-value*
	Casein	Soybean tempe	Velvet bean tempe	Combination tempe	
Moisture (%wb)	4.89±0.35[a]	7.69±0.24[b]	9.39±0.12[c]	7.69±0.24[b]	<0.001
Ash (%db)	4.02±0.02[c]	1.84±0.01[b]	1.32±0.02[a]	1.84±0.01[b]	<0.001
Protein (%db)	78.12±0.24[c]	44.86±0.02[b]	36.77±0.30[a]	43.58±0.41[b]	<0.001
Fat (%db)	0.13±0.00[a]	25.87±0.43[d]	3.94±0.13[b]	24.01±0.28[c]	<0.001
Crude fiber (%db)	0[a]	2.21±0.24[c]	1.30±0.39[b]	1.15±0.30[b]	0.006
Carbohydrate (%db)	14.73±0.21[a]	23.82±0.54[b]	95.24±2.26[d]	29.31±0.92[c]	<0.001

Different letter superscripts (a, b, c, and d) on the same row indicate significant differences
* Analyzed using ANOVA

Each diet was adjusted to achieve a 10% protein level, with velvet bean tempe showing the highest protein amount (273.52 g) and casein the lowest (128.02 g) due to differences in protein density. Corn oil content varied to balance fat requirements, with velvet bean tempe containing 69.21 g and combination tempe the least (24.91 g). Corn starch served as the primary composition, with the highest amount in the casein group and the lowest in the velvet bean tempe group, with 683.57 g and 570.14 g, respectively (**Table 2**).

Table 2. Composition of experimental rat rations based on the protein sources

Ration composition (g/1000g)	Treatment groups based on protein source*				
	Non-protein	Casein	Soybean tempe	Velvet bean tempe	Combination tempe
Protein source	0	128.02	222.92	273.52	229.44
Corn oil	80	79.83	21.21	69.21	24.91
Mineral mix	50	44.85	45.90	46.38	45.82
Vitamin mix	10	10	10	10.00	10.00
Carboxy methyl cellulose (CMC)	10	10	5.08	6.44	7.37
Water	50	43.73	32.86	24.30	31.82
Corn starch	800	683.57	662.04	570.14	650.64

* Each ration contains 10% protein

Cognitive function analysis

A comparative analysis of the travel time parameter required to solve the classic maze test is depicted in **Figure 1**. The results revealed that rats fed with non-protein ration had significantly slower travel times (p=0.001) than those receiving protein-supplemented rations (casein, soybean tempe, velvet bean tempe, and combination tempe). The travel time was not significantly different among groups of rats consuming various protein-containing diets (p=0.061).

Brain mass and volume analysis

The results of measurements of brain mass and volume are presented in **Table 3**. Rats treated with non-protein rations had significantly lighter brain masses (p<0.001) than other treatments. Rats with the combined tempe treatment had brain mass that was not significantly different (p=0.061) from those treated with soybean tempe, casein, and velvet bean tempe. Rats treated with velvet bean tempe had significantly lighter brain mass (p<0.001) than rats treated with casein (**Table 3**).

The analysis of brain volume parameters showed that rats treated with non-protein rations had significantly smaller brain volumes (p<0.001) than those treated with protein (**Table 3**). There was no statistically significant difference in brain volume between treatment groups consuming rations with different types of protein (p=0.337) (**Table 3**).

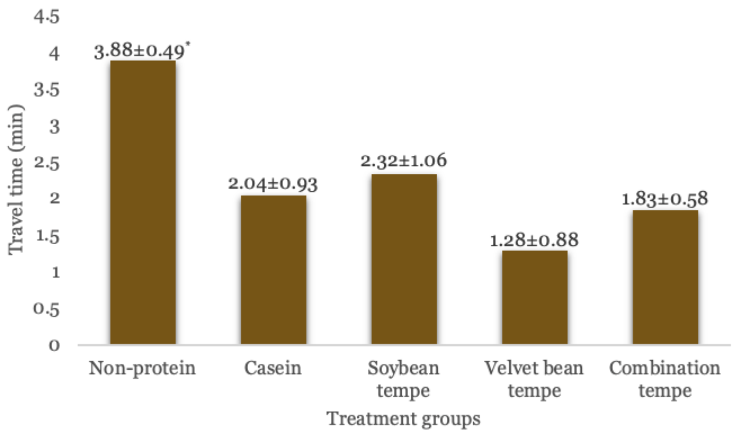

Figure 1. Comparisons of cognitive function analysis based on the time required to complete the classic maze test among groups treated with different protein sources. The symbol of * indicates a significant difference at $p<0.05$ with other groups.

Table 3. Comparison of rats' brain mass and volume among groups treated with different protein sources

Treatment group	Brain mass (g)	Brain volume (mL)
Non-protein	1.63±0.67^{a}	1.40±0.23^{a}
Casein	1.92±0.07^{c}	1.99±0.07^{b}
Soybean tempe	1.89±0.80^{bc}	1.92±0.06^{b}
Velvet bean tempe	1.79±0.07^{b}	1.97±0.05^{b}
Combination tempe	1.85±0.10^{bc}	1.98±0.04^{b}
p-value*	<0.001*	<0.001*

Different letter superscripts (a, b, c, and d) on the same column indicate significant differences.
* Statistically significant at $p=0.05$, using ANOVA

Histological profile of the hippocampus

The number of hippocampal neuron cells in the dentate gyrus section is presented in **Table 4**. The number of neuron cells in the dentate gyrus, CA1 and CA3 regions showed variation across treatment groups, highlighting notable differences between protein sources. In the dentate gyrus, significant differences in neuron number were observed ($p<0.001$), with the velvet bean tempe group having the highest neuron counts (165.85±9.01), followed by soybean tempe (158.30±6.45), combination tempe (151.40±5.93), casein (119.40±6.03), and the non-protein ration (95.12±11.49). In the CA1 region, while differences were not statistically significant, the velvet bean tempe group still presented the highest cell density (67.05±8.80), with other protein groups showing moderate increases over the non-protein ration. Significant differences in the CA3 region ($p<0.001$) also indicated that velvet bean tempe had the highest neuron count (51.40±5.85), followed by combination tempe and soybean tempe (**Table 4**).

Table 4. The number of hippocampus neuron cells among groups treated with different protein sources

Treatment	Number of neuron cells per visual field (400×)		
	Dentate gyrus	Cornu ammonis 1 (CA1)	Cornu ammonis 3 (CA3)
Non-protein	95.12±11.49^{a}	52.75±2.12^{a}	35.25±7.42^{a}
Casein	119.40±6.03^{b}	58.00±8.02^{ab}	37.00±1.97^{a}
Soybean tempe	158.30±6.45^{cd}	61.55±5.88^{ab}	44.40±3.71^{b}
Velvet bean tempe	165.85±9.01^{d}	67.05±8.80^{b}	51.40±5.85^{c}
Combination tempe	151.40±5.93^{c}	64.60±7.89^{ab}	45.30±3.62^{bc}
p-value	<0.001*	0.169	<0.001*

Different letter superscripts (a, b, c, and d) on the same column indicate significant differences.
* Statistically significant at $p=0.05$, using ANOVA

Brain dopamine and serum β-amyloid levels

The levels of dopamine and β-amyloid in rats across various ration treatments are presented in **Table 5**. The analysis revealed significant differences in brain dopamine and serum β-amyloid levels among groups, both had $p<0.001$. The animals in the soybean tempe group had the highest dopamine level at 3.64±0.32 ng/100g, followed by those in casein group at 2.45±0.28 ng/100g and velvet bean tempe at 2.24±0.22 ng/100g. The non-protein ration group had the lowest dopamine level at 1.88±0.31 ng/100g (**Table 5**). Detailed comparisons between the two study groups are presented in **Table 5**. In terms of β-amyloid levels, the non-protein group had the highest concentration at 58.03±6.79 pg/mL, followed by the combination tempe group at 42.61±1.90 pg/mL and casein at 38.07±0.24 pg/mL. The soybean tempe group showed the lowest β-amyloid level at 29.01±5.45 pg/mL (**Table 5**). Detailed comparisons between the two study groups are presented in **Table 5**.

Table 5. Dopamine and β-amyloid levels among groups treated with different protein sources

Treatment	Dopamine (ng/100gr)	β-amyloid (pg/mL)
Non-protein	1.88±0.31[a]	58.03±6.79[d]
Casein	2.45±0.28[b]	38.07±0.24[b]
Soybean tempe	3.64±0.32[c]	29.01±5.45[a]
Velvet bean tempe	2.24±0.22[ab]	50.52±1.25[c]
Combination tempe	2.16±0.08[ab]	42.61±1.90[b]
p-value	<0.001*	<0.001*

Different letter superscripts (a, b, c, and d) on the same column indicate significant differences.
* Statistically significant at p=0.05, using ANOVA

Factors associated with cognitive function

To have a better understanding of factors associated with cognitive function, several variables were measured by combining the data from all rat groups and their correlations with cognitive function were measured using the Pearson correlation test. The analyses found that there were strong negative correlations between brain mass (-0.694), brain volume (-0.894), dentate gyrus neuron counts (-0.833), CA1 neuron counts (-0.731), and CA3 neuron counts (-0.697) with the travel time required to solve the classic maze by the animals, which was used as the cognitive function indicator used in this study. In contrast, dopamine (-0.288) and β-amyloid (0.440) had weak correlations with the travel time (**Table 6**).

Table 6. Pearson correlation analysis showing factors influencing cognitive function

Dependent variable	Pearson correlation coefficient						
	Brain mass	Brain volume	Number of neuron cells			Dopamine	β-amyloid
			Dentate gyrus	CA1	CA3		
Traveling time	-0.694*	-0.894*	-0.833*	-0.731*	-0.697*	-0.288	0.440

CA1: cornu ammonis 1; CA3: cornu ammonis 3.
* Statistically significant at $p<0.05$

Discussion

Velvet bean tempe has encountered consumer resistance primarily due to its undesirable sensory attributes. Despite its potential health benefits, attributed to the presence of L-dopa, a precursor to dopamine, its unappealing taste and appearance have limited its widespread adoption. To address these challenges, the combination of velvet beans with soybeans in tempe production has been explored to enhance its acceptability and nutritional profile. A recent study demonstrated that this combination significantly improved the sensory appeal of the tempe, as assessed through hedonic ratings and rate-all-that-apply (RATA) evaluations, with participants preferring the velvet bean-soybean tempe over its velvet bean-only counterpart, indicating that the addition of soybeans contributed to a more favorable aroma, texture, and color [15]. Moreover, this study revealed that the combination tempe had a higher protein content compared to tempe made solely from velvet beans. This nutritional enhancement is likely due to the synergistic effect of two protein-rich sources, including soybeans and velvet beans. With their high protein content (37–42%) and complete amino acid profile, soybeans provide a valuable nutritional boost to velvet beans, resulting in a tempe product with improved protein quality.

This study investigated the potential cognitive-enhancing effects of soybean-velvet tempe. Using the maze test, the time required for rats to navigate the maze served as a metric for learning and memory performance [3]. In our study, the protein content of the diet significantly influenced cognitive function. A previous study has established a correlation between the quality of dietary protein, particularly the availability of essential amino acids, and enhanced cognitive performance, which includes improved focus, cognitive function, and psychosocial well-being [16]. The fermentation process involved in tempe production has increased nutrient bioavailability by simplifying complex compounds and converting insoluble proteins into soluble forms [17]. These soluble compounds are more readily absorbed and utilized by the organism. The observed enhancement in cognitive function among rats fed rations containing soy, specifically those in the soybean tempe and combination tempe groups, is likely attributed to the presence of isoflavones, primarily daidzein and genistein, in their free aglycone form [11].

Daidzein, in particular, has been shown to enhance cognitive motor function and reduce anxiety behaviors in Parkinson's disease model rats [18]. Similarly, the consumption of genistein has led to improved behavioral outcomes in Alzheimer's disease model rats [19]. The potent antioxidant properties of genistein partially account for these beneficial effects. Additionally, the presence of folic acid and vitamin B12 in soy tempe is believed to contribute to the cognitive improvements observed in these rats. The fermentation process involved in tempe production, which is facilitated by non-pathogenic *Klebsiella pneumoniae*, results in the synthesis of vitamin B12, further enhancing the nutritional quality of this soy-based food [20].

On the other hand, the natural L-dopa content in velvet beans, together with alkaloids and ursodeoxycholic acid, plays an essential role as a neuroprotective agent that helps maintain cognitive function [21]. While synthetic L-dopa is currently used as a therapy for individuals with Parkinson's disease, it can have side effects, including dyskinesia (uncontrolled movements) [22,23]. Additionally, the high antioxidant activity of compounds found in velvet beans further supports its role as a neuroprotective agent [21]. A study indicated that raw velvet bean seed extract can inhibit the activities of acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and monoamine oxidase (MAO), all of which are associated with decreased cognitive function in individuals with Alzheimer's disease [22].

In this study, the brain volume indicated a strong correlation with cognitive function in rats. The protein derived from the three types of tempe is effectively utilized to enhance the brain volume of these rats. This suggests that plant-based protein from soybeans is a viable option for increasing brain mass and volume, potentially leading to improvements in individual intelligence [23]. Furthermore, the evolution of brain size is positively correlated with the increase in the number of neuron cells, which plays a crucial role in enhancing cognitive function [24]. Specifically, a study stated that protein deficiency in rats significantly impacts brain size, dendritic arborization, cell maturation, and brain connectivity that their offspring can inherit [25]. This phenomenon was evident in this study, where rats fed a protein-deficient diet exhibited significantly lower brain mass and volume.

Histological analysis of the hippocampus in this study was conducted to investigate the effects of ration treatment on cellular stages by observing the number of neuron cells in the dentate gyrus, CA1, and CA3 regions of the rat brain's hippocampus. Each part of the hippocampus plays a specific role in regulating cognitive function. Neurons in the dentate gyrus region are crucial for memory formation, significantly contributing to the development of short- and medium-term episodic memories and directly transmitting information to the CA3 region [26]. Neurons in the CA1 region are involved in object recognition, which is essential for navigation and retrieving the essence of events in memory [27,28]. The CA3 region specifically regulates spatial memory, recognition of familiar objects [29], and accuracy in recalling events within a relatively short timeframe [28].

The results indicate that rats in the three types of tempe ration treatment groups had a greater number of neuron cells in the dentate gyrus and CA3 sections, exhibiting a higher density compared to rats treated with rations without protein and casein. These findings suggest that administering soybean tempe, velvet bean tempe, and combination tempe to rats can help preserve the number of neuron cells in the dentate gyrus. This condition points to the presence of bioactive compounds in the three types of tempe, which may protect neuron cells from damage caused by oxidative stress [30]. Oxidative stress can be caused by free radicals, which increase cell death and tissue damage [31].

Diseases associated with cognitive decline are often linked to abnormal levels of two compounds in the brain: dopamine and β-amyloid. A deficiency in dopamine is frequently correlated with the onset of anxiety, difficulties in concentration, and the pathophysiology of Parkinson's disease [34]. Conversely, β-amyloid is commonly recognized as one of the contributing factors to Alzheimer's disease [35]. Interestingly, rats treated with soybean tempe showed the highest dopamine levels and the lowest β-amyloid levels in this study. In contrast, the rats in the velvet bean tempe and combination tempe treatment groups had dopamine levels that were not significantly different from each other. The lower dopamine levels observed in the velvet bean tempe group, compared to the soybean tempe group, may be attributed to the potential loss of L-dopa during the tempe-making process or changes in its form. Additionally, when L-dopa is consumed orally, it is likely decarboxylated into dopamine, preventing it from reaching the central nervous system. Another possibility is that L-dopa is absorbed in the digestive tract of the rats [3].

Conclusion

The quality of protein and the presence of antioxidant compounds in three types of tempe play a crucial role in enhancing cognitive function in experimental rats. Consuming soybean tempe, velvet bean tempe, or a combination of both can improve the cognitive function of rats by preserving neuron cells in the hippocampus, increasing brain mass and volume, elevating dopamine levels, and suppressing serum β-amyloid levels. Notably, the factors that significantly influenced cognitive function were the brain volume and the number of neuron cells in the dentate gyrus. The combined use of soybean and velvet bean tempe demonstrates promising potential for enhancing cognitive function, indicating a synergistic effect between these two bean varieties.

Ethics approval

This analysis had passed ethical review by the Animal Ethics Commission of the School of Veterinary Medicine and Biomedicine, Institut Pertanian Bogor, No. 035/KEH/SKE/III/2023.

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

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References

1. Kala BK, Mohan VR. Nutritional and anti-nutritional potential of three accessions of itching bean (*Mucuna pruriens* (L.) DC var. *pruriens*): An under-utilized tribal pulse. Int J Food Sci Nutr 2010;61(5):497-511.

2. Sardjono RE, Musthapa I, Sholihin H, Ramdhani RP. Physicochemical composition of Indonesian velvet bean (*Mucuna pruriens* L.). Glob J Res Med Plants Indig Med 2012;1(4):101-108.
3. Rima, Ishmayana S, Made Malini D, Soedjanaatmadja UMS. Nutritional content and the activities of L-dopa (L-3,4-dihydoxyphenyalanine) from *Mucuna pruriens* L. DC seeds of Central Java accession. Arab J Chem 2023;16(1):104390.
4. Salsabila SF, Aligita W, Mulyani Y. Review: Neuroprotective effect of herbal plant extracts against Parkinson's disease. Sci J Pharm 2021;17(2):197-209.
5. Cilia R, Laguna J, Cassani E, et al. Daily intake of *Mucuna pruriens* in advanced Parkinson's disease: A 16-week, noninferiority, randomized, crossover, pilot study. Parkinsonism Relat Disord 2018;49:60-66.
6. Astawan M, Faishal MA, Prayudani APG, *et al*. Effects of seed germination on physicochemical and bioactive compounds characteristics of velvet bean tempe. Curr Res Nutr Food Sci 2023;11(2):808-821.
7. Kadar AD, Astawan M, Putri SP, *et al*. Metabolomics based study of the effect of raw materials to the end product of tempe—an Indonesian fermented soybean. Metabolites 2020;10(9):1-11.
8. Astawan M, Wresdiyati T, Subarna, *et al*. Calcium bioavailability of tempe and boiled soybean flours and its effect on osfemurs in experimental rats. J Nutr Sci Vitaminol 2020;66:S314-S319.
9. Association of Official Analytical Chemistry. Official Method of Analysis. 19th ed. Gaithersburg: The AOAC, inc; 2012.
10. Kridawati A, Damanik R, Hardinsyah H, *et al*. Tempe and tofu flour may have positive effect on cognitive function. Ann Nutr Metab 2013;63:2013-2014.
11. Wresdiyati T, Werdhiwati P, Astawan M. The profile of spermatogenic cells and superoxide dismutase in the testis of rats under boiled grobogan tempe and soybean flour treatment. IOP Conf Ser Earth Environ Sci 2018;196(1):012031.
12. Alfarisi H, Subangkit M, Sa'diah S, Wresdiyati T. Acute toxicity test of ethanolic extract of *Acalypha hispida* leaves in female rats: A physiological and histological study. J Kedokt Hewan - Indones J Vet Sci 2020;14(3):48-53.
13. Msibi ZNP, Mabandla MV. Oleanolic acid mitigates 6-hydroxydopamine neurotoxicity by attenuating intracellular ROS in PC12 cells and striatal microglial activation in rat brains. Front Physiol 2019;10:1059.
14. Fitri SMA, Astawan M, Nurtama B, *et al*. Sensory profile of tempe made from a combination of velvet bean and soybean using rate-all-that-apply. Food Res 2024;8 Suppl 4:20-32.
15. Suzuki H, Yamashiro D, Susumu O, *et al*. Intake of seven essential amino acids improves cognitive function and psychological and social function in middle-aged and older adults: A double-blind, randomized, placebo-controlled trial. Front Nutr 2020;7:586166.
16. Astawan M, Wresdiyati T, Yoshari RM, *et al*. The physicochemical properties of tempe protein isolated from germinated and non-germinated soybeans. J Nutr Sci Vitaminol (Tokyo) 2020;66(Supplement):S215-S221.
17. Wang X, Yin Z, Meng X, *et al*. Daidzein alleviates neuronal damage and oxidative stress via GSK3β/Nrf2 pathway in mice. J Funct Foods 2022;92:105060.
18. Mas-bargues C, Borr C, Vina J. The multimodal action of genistein in Alzheimer's and other age-related diseases. Free Radic Biol Med 2022;183:127-137.
19. Abdurrasyid Z, Astawan M, Lioe HN, Wesdiyati T. Physicochemical and antioxidant properties of germinated soybean tempe after two days additional fermentation time. Biointerface Res Appl Chem 2023;13(3):238.
20. Rachsee A, Chiranthanut N, Kunnaja P, *et al*. *Mucuna pruriens* (L.) DC. seed extract inhibits lipopolysaccharide-induced inflammatory responses in BV2 microglial cells. J Ethnopharmacol 2021;267:113518.
21. Kamkaen N, Chittasupho C, Vorarat S, *et al*. *Mucuna pruriens* seed aqueous extract improved neuroprotective and acetylcholinesterase inhibitory effects compared with synthetic L-dopa. Molecules 2022;27(10):3131.
22. Meder D, Herz DM, Rowe JB, *et al*. The role of dopamine in the brain - lessons learned from Parkinson's disease. Neuroimage 2019;190:79-93.
23. Adefegha SA, Oboh G, Oyeleye SI, *et al*. Cognitive enhancing and antioxidative potentials of velvet beans (*Mucuna pruriens*) and horseradish (*Moringa oleifera*) seeds extracts: A comparative study. J Food Biochem 2017;41(1):1-11.
24. Vandenplas Y, Hegar B, Munasir Z, *et al*. The role of soy plant-based formula supplemented with dietary fiber to support children' s growth and development: An expert opinion. Nutrition 2021;90:111278.
25. Marhounová L, Kotrschal A, Kverková K, *et al*. Artificial selection on brain size leads to matching changes in overall number of neurons. Evolution 2019;73(9):2003-2012.
26. Cormack BE, Harding JE, Miller SP, *et al*. The influence of early nutrition on brain growth and neurodevelopment in extremely preterm babies: A narrative review. Nutrients 2019;11(9):1-24.
27. Tilger MADS, Gaiardo RB, Cerutti SM. Inactivation of the dorsal CA1 hippocampus impairs the consolidation of discriminative avoidance memory by modulating the intrinsic and extrinsic hippocampal circuitry. J Chem Neuroanat 2023;128:102209.

28. Nagelhus A, Andersson SO, Cogno SG, *et al*. Object-centered population coding in CA1 of the hippocampus. Neuron 2023;111(13):2091-2104.e14.
29. Atucha E, Ku S, Lippert MT, Sauvage MM. Recalling gist memory depends on CA1 hippocampal neurons for lifetime retention and CA3 neurons for memory precision. Cell Rep 2023;42(11):113317.
30. Pimentel GA, Crestani AM, Florindo LH. Do spatial and recognition memories have a lateralized processing by the dorsal hippocampus CA3?. Behav Brain Res 2022;416:113566.
31. Osuntokun OS, Olayiwola G, Oriare AK, *et al*. *Mucuna pruriens* seed protects the hippocampal neurons and abrogates seizure indices in chemically-convulsed mice: Evidence of the Nrf2 expression defense pathway. J Chem Neuroanat 2022;123:102115.
32. Khosravi A, Razavi SH. Therapeutic effects of polyphenols in fermented soybean and black soybean products. J Funct Foods 2021;81:104467.
33. Liu X, Cheng ZY, Li YF, *et al*. Dopamine D2 receptor agonist bromocriptine ameliorates Aβ1-42-induced memory deficits and neuroinflammation in mice. Eur J Pharmacol 2023;938:175443.
34. Chen GF, Xu TH, Yan Y, *et al*. Amyloid beta: Structure, biology and structure-based therapeutic development. Acta Pharmacol Sin 2017;38:1205-1235.