International Journal of Agriculture Innovations and Research
Volume 12, Issue 5, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy):
Received: 05/03/2024 | Accepted on: 25/03/2024 | Published: 08/04/2024

Acetylcholinesterase Activity in the Brain Regions of Pubertal Pigs Fed Dietary Neem (*Azadirachta indica* A. Juss) Kernel

Olujide Adedamola Sokunbi[1*], Jovi Richard Otite[2], John Olusoji Abiola[3] and Olufemi Alaba[1]
[1]Animal Physiology and Bioclimatology Unit, Department of Animal Science, University of Ibadan, Nigeria.
[2]Assisted Reproduction Technology Unit, Teaching and Research Farm, University of Ibadan, Nigeria.
[3]Department of Veterinary Medicine, University of Ibadan, Nigeria.
*Corresponding author email id: jide.sokunbi@gmail.com

Abstract **– Neem kernel (NK) with a higher dietary energy than maize could be a viable ingredient in swine nutrition. However, there are established documentation of many chemical constituents in neem that are classified as antinutrients. Hence, the effects of dietary NK on acetylcholinesterase (AChE) activities in the brain regions of pubertal boars and pigs were investigated. Acetylcholinesterase is the enzyme which catalyses the hydrolysis of acetylcholine and is known to mediate food and water intake. A feeding trial was conducted with forty growing pigs consisting of twenty males and twenty females (8 to 10 weeks old) in a completely randomised design, to evaluate the influence of NK inclusion in swine diets at 0, 50, 100 and 150 g/kg, on AChE activities in the brain regions of pigs. The pigs were raised until they attained pubertal age and were subsequently sacrificed. The AChE activity (µmole/g/ minute) was determined by colorimetric method. Data were analysed using descriptive statistics and ANOVA at α 0.05 Pigs on 150 g/kg NK diet showed significantly (P<0.05) reduced AChE activities in cerebrum, cerebellum, mesencephalon, pons varolii, and medulla oblongata compared to those on control. Significant (P< 0.05) increase in AChE activity was, however, observed for hypothalamus. Neem kernel can be included up to 100 g/kg in swine diets without deleterious effects on the AChE activities in the brain regions of growing pigs. Also, results suggested that effects of NK on AChE activities were more pronounced in pubertal gilts than in boars.**

Keywords **– Neem Kernel, Acetylcholinesterase Activity, Brain Regions, Growing and Pubertal Pigs.**

I. INTRODUCTION

Acetylcholinesterase (AChE) is one of the enzymes in the brain and the nervous system that is considered to be of fundamental importance to the chemical integration of the animal (Adejumo, 1980) as it plays a crucial role in maintaining the delicate balance of signals controlling our muscles, thoughts, and emotions (Rajagopalan *et. al.,* 2023). Acetylcholinesterase can also be linked in the pathogenesis of Autism (Ure *et. al.,* 2023) and Alzheimer disease (Rees and Brimijoin, 2003) as it facilitates nervous transmission and mediates behavioural responses such as sexual behaviour, aggressive behaviour, basic reflexes, mental coordination, and muscular contraction (Frandsen *et. al.,* 2009). Its influence is mainly based on its ability to hydrolyse acetylcholine (McHardy *et. al.,* 2017), responsible for the above physiological actions. Some of the factors affecting AChE activity are inhibitors, temperature, hormones, pH, age, and nutrition (Adejumo, 1980). Of importance is nutrition, and its interaction with inhibitors, hormones, and concentration of (AChE).

Bass *et al*. (1970) reported that pigs maintained post-natal on protein restricted diet indicated severe growth retardation and impaired brain development and there was also a significant decrease in total brain AChE activity compared to the control. However, they observed increase in Specific AChE (SAChE) activity in the cerebral cortex, compared to the control. These observations suggest a close relationship between rate of growth and AChE activity. Im *et al* (1973) reported elevated AChE activity in pigs that had experienced protein-calorie deprivation early in life. They also observed increased specific AChE activity in brain of nutritionally deprived

animals over the control animals. The cerebrum was indicated to be very sensitive to malnutrition while the cerebellum and brain stem appeared to be unaffected. Evidence abounds that brain transmitters especially of the cholinergic pathway are deeply involved in ovarian functions and hormone action. The interaction between acetylcholinesterase (AChE) activity of the brain regions of gilts and gonadal hormones are also fairly documented (Adejumo and Egbunike, 2002).

Neem (*Azadirachta indica*) seeds contain the chemical azadirachtin (Nagini *et al.,* 2023) and is used as an active ingredient in various biopesticide. Annongu *et. al*. (2003a) observed reduced growth rate in pigs when fed untreated neem seed. This could be as a result of AChE's ability to degrade acetylcholine (Mladenovic, 2018), which is responsible for proper distribution of food as well as an important neurotransmitter in the central nervous system of mammals and insects (Jones, 2005). Azadirachtin has a great influence in the AChE activity. Acetylcholine is produced by the synthetic enzyme cholineacetyl transferase, which uses acetyl co-enzyme A and choline as a substrate for the formation of acetylcholine. Although acetyle choline is also synthesized in the brain, dietary choline is a major source of acetylcholine as it supplies the needed free choline required for acetylcholine synthesis (Wurtman et al., 2009; Wiedeman et. al., 2018). Acetylcholine is readily hydrolysed to choline and acetic acid by the action of the enzyme acetylcholinesterase, found not only at the nerve ending but also within the nerve fibre. The action of Acetylcholine in the body is controlled by the inactivating effect of acetylcholinesterase. Inhibition of acetylcholinesterase with resultant prolongation of parasympathetic activity is affected by physostigmine and the action is reversible. Neostigmine is a water-soluble alkaloid which is thought to function also as an inhibitor of acetylcholinesterase (Neely *et al.,* 2023) and thus to prolong acetylcholine or parasympathetic action (Si Shangkun *et al.* 2023). A synthetic compound diisopropylfluorophosphate, also inhibits the esterse activity but in an irreversible manner. A mechanism for detoxifying diispropylfluorophosph--ate exists in the body, in the action of an enzyme capable of bringing about hydrolysis of the compound to fluoride and diisopropylphosphate. This enzyme, diisopropylfluorophosphatase, has been identified in the kidney and is activated by Mn^{2+} and Co^{2+} and specific cofactors such as imidazole and pyrrolidine derivatives (for example, proline or hydroxyproline). A number of anticholineterases similar in their action to diisopropylfluorophosphate have been investigated. They serve as the active principles of insecticides. Atropine is used as an antidote to the toxic effects of diisopropylfluorophosphate and other anticholinestereses (Vitorović-Todorovic and Vujatovic-Velimirov, 2023).

The brain by virtue of its position in the central nervous system plays a modulating role on all body functions and through its control on the hypophyseal hormonal pathways keeps the level of hormonal production and metabolism of the other glands normal (Adejumo and Egbunike, 2002). Physiological studies have revealed that brain neurotransmitters particularly of the cholinergic pathway are of vital importance to the physiological integrity and therefore productivity of the animal (Reis *et al*., 1977). The neurotransmitters are selectively concentrated in the different regions of the brain depending on the functions of the regions (Strazielle *et al*., 1998). The high-energy requirement of most portions of the brain is related to the transport of ions, the synthesis of acetylcholine and the metabolism of glutamic acid (Reis *et al*., 1977). The transmitter substance at the majority of synapses is acetylcholine and is believed to be the mediator of all synapses between preganlionic and postganglionic fibres of the autonomic nervous system, parasympathetic and sympathetic endings. Acetylcholinesterase is the enzyme which catalyses the hydrolysis of acetylcholine and is known to mediate food and water intake (Adams, 1973, Adejumo and Egbunike, 1988). It modulates aggressive sexual behaviour,

sexual development and is affected by the presence or absence of sex hormones such as estradiol (Packard *et al.* 1996). The mode of action is generally believed to be through its concentration and therefore rate of hydrolyses of acetylcholine (Adejumo and Egbunike, 2002).

Drugs and probably antinutrients could act at many places to interfere with the ACh synapse. They could block the synthesis of ACh, its transport down the axon, its formation into vesicles, its release into the synapse, its attachment to the receptor or its breakdown by AChE (Thompson, 1985). The aim of this investigation is to observe the activity of Acetylcholinesterase in different regions of the brain of pubertal boars and gilts.

II. MATERIALS AND METHODS

The Experimental Diets

The composition of the experimental diets formulated for this study is presented in Table 1.

Table 1. Composition of experimental diets.

Ingredients (Kg)	**Dietary neem kernel (g/kg)**			
	0	50	100	150
Maize	40.00	35.00	30.00	25.00
Neem kernel	0.00	5.00	10.00	15.00
Groundnut cake	15.00	15.00	15.00	15.00
Palm kernel cake	10.00	10.00	10.00	10.00
Wheat offal	13.00	13.50	14.00	15.00
Corn bran	15.00	15.00	15.00	15.00
Fish meal (65 %)	2.00	2.00	2.00	2.00
Bone meal	1.00	1.00	1.00	1.00
Oyster shell	0.50	0.50	0.50	0.50
Salt	0.40	0.40	0.40	0.40
Premix*	0.50	0.50	0.50	0.50
Lysine	0.30	0.30	0.30	0.30
Methionine	0.30	0.30	0.30	0.30
Vegetable oil (Palm oil)	2.00	1.50	1.00	0.00

*Micro-Mix Growers: 2.5 kg of premix contains Vitamin A (10,000,000.00 I.U.); Vitamin D_3 (2,000,000.00 I.U.); Vitamin E (20,000.00 mg); Vitamin K_3 (2,000.00 mg); Vitamin B_1 (3,000.00 mg); Vitamin B_2 (5,000.00 mg); Niacin (45,000.00 mg); Calcium Pantothenate (10,000.00 mg); Vitamin B_6 (4,000.00 mg); Vitamin B_{12} (20.00 mg); Folic Acid (1,000.00 mg); Biotin (50.00 mg); Choline Chloride (300,000.00 mg); Manganese (120,000.00 mg); Iron (100,000.00 mg); Zinc (80,000.00 mg); Copper (8,500.00 mg); Iodine (1,500 mg); Cobalt (300.00 mg); Selenium (120.00 mg); Anti-Oxidant (120,000.mg).

Animals, Experimental Design, and Management

Forty growing pigs consisting of twenty males and twenty females were used for this study. The experimental design, feeding and management were as earlier described in previous studies.

Brain Sampling

The pigs were raised until they attained pubertal age and were subsequently sacrificed. Prior to slaughter the pigs were starved for 18 hours but allowed free access to drinking water. They were then weighed, exsanguinated, bled rapidly and the heads quickly severed from the rest of the bodies. The heads were quickly sawn open and the brains were carefully removed in toto from the skull case. Brains were immediately freed of adhering meninges and superficial blood vessels or clots, weighed and dissected on an ice-cold porcelain tile as earlier described by Egbunike (1981) into eight areas namely, cerebrum, cerebellum, mesencephalon, pons varolii, medulla oblongata, amygdala, hippocampus, and hypothalamus. Samples from these eight areas of the brain were quickly frozen at -20 ºC until processing which was not more than 60 minutes afterwards to ensure minimal loss of enzyme activity. All brain samples were macerated at a dilution rate of 10 mg of tissue to 1 ml of 0.1 M ice-cold phosphate buffer (pH 7.4).

Determination of AChE Activity

AChE activity was determined by colorimetric method of Ellman *et. al.* (1961) which measures the rate of hydrolysis of acetylcholine iodide substrate to thiocholine and acetate using 5, 5 - dithiobis - 2 - nitrobenzoate (DTNB) as the colour reagent. AChE activity was read using an Eppendorf photometer 1101 M at 405 nm and then expressed in µmole/gram wet tissue/minute.

Determination of Total Protein

Total Protein was determined using the Biuret method as described by Ritzmann and Daniels (1979). The concentration of Total Protein was read using an Eppendorf photometer 1101 M at 564 nm and then expressed in g/dl.

Determination of Specific AChE activity (SAChE)

The AChE activity of the brain sample was divided by its total protein concentration to give the specific activity and is expressed in µmole/gram protein/minute.

Statistical Analyses

Results were subjected to statistical analysis using the analysis of variance procedure of statistical analysis software (SAS, 2016). The treatment means were presented with group standard errors of means and where significant, were compared using the Duncan procedure of the same software.

III. Results and Discussion

Results

Regional variations of acetylcholinesterase activity, total protein, and specific acetylcholine esterase activity in the brain of pubertal boars fed dietary neem kernel.

The profiles of the AChE activity, total protein concentration and SAChE activity distribution in the brain regions of peripubertal male pigs are presented in Tables 2, 3 and 4 respectively. AChE activity was significant ($P<0.05$) between experimental diets for cerebrum, cerebellum, hippocampus, and hypothalamus. Similarities in values were observed for mesencephalon, pons varolii, medulla oblongata and amygdala. The discernable trend

where significant differences in activities were observed is the increase in activity at 50 g/kg NK diet and a reduction at 150 g/kg NK diet except for hypothalamus, where NK in swine diets elicited an increase in activity as the level of its inclusion increased.

Total protein concentration values were similar in cerebrum, cerebellum, and amygdala, while significant (P< 0.05) differences in values were observed for mesencephalon, pons varolii, medulla oblongata, hippocampus, and hypothalamus. Total protein concentration dropped significantly (P<0.05) at 50 g/kg NK diet and normalized at 100 and 150 g/kg NK diets for mesencephalon and pons varolii while mean values observed for medulla oblongata, hippocampus and hypothalamus increased as the level of NK in Swine diet increased.

SAChE activity was significantly (P<0.05) different between experimental diets for cerebrum, cerebellum, medulla oblongata, hippocampus, and hypothalamus (P<0.05). The observed general trend in all these brain regions is a linear decrease in SAChE activity with increase in NK concentration except for hypothalamus where the reverse occurred. Results showed non-significance between mean SAChE activity for mesencephalon, pons varolii and amygdala.

Table 2. Regional variations of acetylcholinesterase activity in the brain of pubertal boars fed diets containing neem kernel.

Parameters	**Dietary Neem Kernel (g/kg)**				**Group SEM**
	0	**50**	**100**	**150**	
Initial Live Wt (kg)	11.20	11.25	11.30	11.35	0.18
Final Live Wt (kg)	38.29^{a}	40.34^{a}	33.56^{b}	29.55^{c}	0.98
Brain Wt (g)	90.59^{a}	91.55^{a}	90.40^{a}	94.36^{b}	0.57
Relative Wt of Brain	0.24^{a}	0.23^{a}	0.27^{b}	0.32^{c}	0.05
†*AChE Activity*					
Cerebrum	1.08^{a}	1.12^{a}	1.12^{a}	0.66^{b}	0.07
Cerebellum	2.70^{a}	2.55^{ab}	2.45^{b}	2.41^{b}	0.04
Mesencephalon	3.42	3.43	3.64	3.85	0.12
Pons Varolii	2.59	2.40	2.26	2.55	0.11
Medulla Oblongata	3.01	2.95	2.92	2.89	0.05
Amygdala	3.71	3.57	3.86	4.18	0.18
Hippocampus	2.66^{a}	2.70^{a}	2.51^{b}	2.51^{b}	0.29
Hypothalamus	2.96^{a}	3.01^{ab}	3.60^{bc}	3.75^{c}	0.12

SEM = standard error of the mean, NK = neem kernel, Wt = Weight, and abc = means in the same row with superscript differ significantly (P<0.05). † = AChE activity is expressed as micromoles acetylthiocholine iodide hydrolysed per gram wet tissue per minute.

Table 3. Regional variations of total protein concentration in the brain of pubertal boars fed diets containing neem kernel.

Parameters (g/dl)	**Dietary Neem Kernel (g/kg)**				**Group SEM**
	0	**50**	**100**	**150**	
Cerebrum	0.129	0.130	0.128	0.129	0.001
Cerebellum	0.121	0.121	0.121	0.120	0.001

Parameters (g/dl)	Dietary Neem Kernel (g/kg)				Group SEM
	0	50	100	150	
Mesencephalon	0.122[a]	0.107[b]	0.121[a]	0.120[a]	0.002
Pons Varolii	0.114[a]	0.108[b]	0.115[a]	0.119[a]	0.001
Medulla Oblongata	0.107[a]	0.115[b]	0.120[b]	0.119[b]	0.002
Amygdala	0.126	0.126	0.125	0.126	0.001
Hippocampus	0.125[a]	0.127[ab]	0.129[b]	0.128[b]	0.001
Hypothalamus	0.125[a]	0.128[b]	0.129[b]	0.129[b]	0.001

SEM = standard error of the mean, NK = neem kernel and a, b, c = means in the same row with different superscript differ significantly (P < 0.05).

Table 4. Regional variations of specific acetylcholinesterase activity in the brain of pubertal boars fed diets containing neem kernel.

Parameters (µmole/g Protein/Minute)	Dietary Neem Kernel (g/kg)				Group SEM
	0	50	100	150	
Cerebrum	8.31[a]	8.55[a]	8.75[a]	5.11[b]	0.49
Cerebellum	22.30[a]	21.06[ab]	20.31[b]	20.04[b]	0.30
Mesencephalon	27.94	28.30	30.22	32.02	0.88
Pons Varolii	22.51	22.31	19.61	21.46	0.85
Medulla Oblongata	28.09[a]	25.71[b]	24.39[b]	24.23[b]	0.50
Amygdala	29.42	28.29	30.83	33.10	0.88
Hippocampus	21.23[a]	21.37[a]	19.46[b]	19.57[b]	0.24
Hypothalamus	23.77[a]	23.57[a]	27.83[ab]	29.10[b]	0.89

SEM = standard error of the mean, NK = neem kernel and abc = means in the same row with different superscript differ significantly (P<0.05).

Regional Variations of acetylcholinesterase activity, Total protein concentration and specific Acetylcholinester--ase activity distribution in the Brain of pubertal gilts fed dietary neem kernel.

The profiles of the AChE activity, total protein concentration and SAChE activity distribution in the brain regions of peripubertal female pigs are presented in Tables, 5, 6 and 7 respectively. AChE activity was significant (P<0.05) in all brain regions except for hippocampus, between experimental diets. The discernible trend is a reduction in activity with increasing concentration of NK in swine diets for all brain regions except for hypothalamus.

Total protein concentration values were similar in mesencephalon, medulla oblongata, amygdala, and hippocampus, while significant (P<0.05) differences in values were observed for cerebrum, cerebellum, pons varolii and hypothalamus. Total protein concentration dropped significantly (P<0.05) from 50 g/kg NK diet for cerebrum and cerebellum while there was significant (P<0.05) increase in total protein concentration from 50 g/kg NK diet for pons varolii and hypothalamus.

Specific acetylcholinesterase activity was significantly different (P<0.05) between experimental diets for all brain regions except for hippocampus where observed difference in mean SAChE activity were non-significant. A different picture of SAChE activity from male pigs was presented by the results for female pigs in that the initial reduction in activity from 50 g/kg NK diet to 100 g/kg NK diet was reversed at 150 g/kg NK diet for cerebrum, mesencephalon, pons varolii, amygdala and hypothalamus. However, this recovery is not evident for cerebrum, cerebellum, medulla oblongata and hippocampus.

Table 5. Regional variations of acetylcholinesterase activity in the brain of pubertal gilts fed diets containing neem kernel.

Parameters	**Dietary Neem Kernel (g/kg)**				**Group SEM**
	0	**50**	**100**	**150**	
Initial Live Wt (kg)	10.80	10.74	10.78	10.86	0.15
Final Live Wt (kg)	40.14^{a}	41.50^{a}	33.90^{b}	31.30^{b}	1.09
Brain Wt (g)	108.94^{a}	111.22^{a}	96.11^{b}	96.96^{b}	1.66
Brain Relative Wt (%)	0.27^{a}	0.27^{a}	0.29^{ab}	0.31^{b}	0.05
†*AChE Activity*					
Cerebrum	1.50^{a}	1.39^{a}	1.02^{b}	0.99^{b}	0.06
Cerebellum	2.13^{a}	1.49^{b}	1.38^{b}	1.52^{b}	0.10
Mesencephalon	2.60^{a}	2.61^{a}	2.01^{ba}	2.26^{a}	0.09
Pons Varolii	2.44^{a}	2.36^{a}	1.80^{ba}	2.07^{a}	0.13
Medulla Oblongata	2.80^{a}	1.84^{b}	1.11^{b}	1.11^{b}	0.16
Amygdala	2.88^{a}	2.45^{b}	2.44^{b}	2.78^{a}	0.09
Hippocampus	2.72	2.40	2.32	2.27	0.14
Hypothalamus	2.58^{a}	2.30^{a}	2.37^{a}	2.92^{b}	0.08

SEM = standard error of the mean, NK = neem kernel, Wt = Weight, and abcd = means in the same row with different superscript differ significantly (P<0.05). † = AChE activity is expressed as micromoles acetylthiocholine iodide hydrolysed per gram wet tissue per minute.

Table 6. Regional variations of total protein concentration in the brain of pubertal gilts fed diets containing neem kernel.

Parameters (g/dl)	**Dietary Neem Kernel (g/kg)**				**Group SEM**
	0	**50**	**100**	**150**	
Cerebrum	0.127^{a}	0.123^{b}	0.124^{b}	0.123^{b}	0.001
Cerebellum	0.125^{a}	0.122^{c}	0.124^{ab}	0.123^{b}	0.001
Mesencephalon	0.125	0.124	0.125	0.124	0.001
Pons Varolii	0.120^{a}	0.122^{b}	0.122^{b}	0.122^{b}	0.001
Medulla Oblongata	0.127	0.126	0.126	0.126	0.001
Amygdala	0.129	0.128	0.128	0.128	0.001
Hippocampus	0.126	0.126	0.126	0.126	0.001
Hypothalamus	0.124^{a}	0.127^{b}	0.127^{b}	0.128^{b}	0.001

SEM = standard error of the mean, NK = neem kernel and abc = means in the same row with different superscript differ significantly (P <0.05).

Table 7. Regional variations of specific acetylcholinesterase activity in the brain of pubertal gilts fed diets containing neem kernel.

Parameters (g/dl)	Dietary Neem Kernel (g/kg)				Group SEM
	0	50	100	150	
Cerebrum	11.74[a]	11.89[a]	8.27[b]	8.05[b]	0.54
Cerebellum	17.03[a]	12.27[b]	11.11[b]	12.34[b]	0.81
Mesencephalon	20.91[a]	21.07[a]	16.06[b]	18.22[a]	0.74
Pons Varolii	20.29[b]	19.32[ba]	14.70[ab]	16.98[b]	0.85
Medulla Oblongata	22.07[a]	14.54[b]	8.78[b]	8.79[b]	1.48
Amygdala	22.38[a]	19.17[b]	19.05[b]	21.83[a]	0.52
Hippocampus	21.54	19.06	18.41	18.02	0.60
Hypothalamus	20.78[a]	18.10[b]	18.70[b]	22.81[a]	0.58

SEM = standard error of the mean, NK = neem kernel and abc = means in the same row with different superscript differ significantly (P<0.05).

IV. Discussion

A look at the regional variation in the AChE and SAChE of experimental pigs (male or female) offered control diet in this study supports the generalised classification of the mesencephalon, medulla oblongata and amygdala as high activity regions; hypothalamus, hippocampus, and pons varolii into medium activity regions; and the cerebrum and cerebellum as low activity regions (Adejumo and Egbunike, 2002).

Inclusion of NK in swine diets appears to generally cause an increase in AChE at 50 and 100 g/kg NK diets for three specific regions of the brain (cerebrum, cerebellum, and hippocampus) for peripubertal boars. This observation did not however hold true for peripubertal gilts, where the inclusion of NK in swine diets caused a depression in AChE from 100 g/kg NK diet from the mesencephalon, pons varolii and amygdala. Depressions from 50 g/kg NK diet were also observed for cerebrum and medulla oblongata. Results also suggested that the effects of NK on AChE were more pronounced in peripubertal gilts than in peripubertal boars. There exists anatomical sex differences in the brain anatomy and this increases during adolescence (Kurth *et al.*, 2021). These differences are influenced by biological and environmental determinants (Ristori *et al*., 2020). Ryan and Arnold (1979) reported evidence of sexual differences in topographical distribution of AChE in the brain.

A relationship between the time difference observed in adjusting to the bitter taste of NK in swine diets and the regional variation of AChE could also be a contributing factor. Many factors control hunger and feeding, including neurons in the hypothalamus that are sensitive to blood glucose, or blood sugar level, whether the stomach is empty or full, the taste of foods, or the time of day. The intestinal tract functions as an endocrine gland, in that it releases several peptide hormones into the blood (Brown and Hazen, 2015). At least two of these, bombesin and cholecystokinin, induce eating behaviour in animals if they are injected into the brain. It seems likely that these “gut hormones” are important in the control of hunger and feeding behaviour (Keisuke *et. al*., 2011).

However, other factors metabolic in origin could also be responsible. For example, glucose is the primary energy substrate of the brain and acts as a precursor to a host of metabolites, some of which are neurotransmitte--rs that have been reported to affect gonadotropin secretion. These include gamma-amino butyric acid, glutamate, and aspartate. Plasma substrates and insulin also affect pituitary function (Adashi *et al.,* 1981). Also, plasma glucocorticoid concentrations are affected by nutritional deficits (Dubey *et al.,* 1986; Dallman *et. al.,* 2004) although exceptions are also documented (Britt *et al.,* 1988). This in turn could lead to a diabetic-like state due to prolonged exposure to glucocorticoids due to increase in plasma glucose (McKay and Cidlowski, 2003). There is also indirect evidence to suggest that adrenal steroids inhibit hypothalamic GNRH release in pigs (Fonda *et al.,* 1984). Furthermore, the blood concentration of choline apparently determines the synthesis of the neurotransmitter acetylcholine in the brain (Sawada *et al*. 1999). It appears that, nutrition directly controls this neurotransmitter, which is known to be involved in the regulation of gonadotropin secretion. Therefore, it would be important to know the effect of NK on choline availability, metabolism and utilization by subjects fed neem.

Neem kernel is reported to be very rich in Glutamic acid (Annongu *et al.*, 2003b). The concentration of this amino acid is about 30 % of the total amino acid content of neem kernel (Annongu *et al*., 2003a). Certain amino acids are believed to be the 'workhorse' fast transmitters in the brain. Some of them seem to always be excitatory, for example, glutamic acid and aspartic acid, and others to always be inhibitory, such as gamma amino butyric acid (GABA) and glycine. The case for glutamic acid is perhaps the strongest of the suspected excitatory amino acid transmitters. At very low doses, Glutamic acid always causes excitation when applied to neurons. It is present in the highest concentrations in the brain. GABA is synthesized from the amino acid L-glutamic acid, a component of natural protein foods. L-glutamic acid is converted into GABA by the enzyme glutamic acid decarboxylase (GAD). The availability of GAD is believed to be the rate-limiting factor in the synthesis of GABA.

The AChE in both young boars and gilts is similar in the hypothalamus. Even at 150 g/kg NK diet, an increased AChE was observed over that in the control diet. Considering the absolute and relative weights of the brain (Tables 2 and 5), it suggests that neem kernel appears to elicit an increase in brain mass. Increase in total protein concentration and SAChE were also indicated for this region of the brain. The roles of the hypothalamus in the control of the gonads are well established. Evidence abounds that brain transmitters especially of the cholinergic pathway are deeply involved in ovarian functions and hormone action. The interaction between acetylcholinesterase activity of the brain regions of gilts and the gonadal hormones are also fairly documented (Adejumo and Egbunike, 2002).

IV. Conclusion

It appears that sex plays an important role on the effects of Neem Kernel in the diets of peripubertal pigs. The effects of neem kennel vary with the region of the brain. While in pubertal males it increases AChE acivity in the cerebrum, cerebellum, and hippocampus, in pubertal females it reduces AChE activity in the cerebrum and medulla oblongata, thus confirming the existence of anatomical differences in the brains of male and female pigs as they attain puberty. On the other hand, the effects of neem kernel on AChE activity increases in the hypothalamus irrespective of sex. Neem kernel appears to elicit an increase in brain mass in pigs on 150 g/kg diet. This work suggests that neem seed can be used as a good energy source in the diets of growing pigs with an

inclusion up to 100g/kg.

REFERENCES

[1] Adams, P.M. (1973). The effect of cholinocytic drugs and cholinesterase blockage on deprivation-based activity and appetite behaviour. *Neuropharmacology.* 12: 825-833.
[2] Adashi, E.Y., Hsueh, A.J.W. and Yen, S.S.C. (1981). Insulin enhancement of luteinizing hormone and follicle stimulating hormone release by cultured pituitary cells. *Endocrinology*. 108: 1441-1449.
[3] Adejumo, D.O. (1980). Changes in acetylcholinesterase activity in the brain and hypophyses of the West African indigenous pig with approaching senility. *M.Sc. Project*. Department of Animal Science. University of Ibadan.
[4] Adejumo, D.O. and Egbunike, G.N. (1988). Effect of thermal stress and water deprivation on the acetylcholinesterase activity of the pig brain and hypophyses. *Int. J. Biometeorol*. 32: 108-111.
[5] Adejumo, D.O. and Egbunike, G.N. (2002). Regional variation in acetylcholinesterase activity and total protein in the brain and hypophyses of Large White boars managed under a hot humid environment. *ASSET series A*. 2 (1): 49-53.
[6] Annongu, A.A., Meuleu, U.ter., Liebert, F., Atteh, J.O. and Joseph, J.K. (2003a). Effects of detoxification on Nigerian neem kernel composition and its impact on swine performance. *Tropical Journal of Animal Science*. 6(1): 137-143.
[7] Annongu, A.A., Meuleu, U.ter, Liebert, F., Atteh, J.O. and Joseph, J.K. (2003b). Detoxification characteristics of dietary neem kernel meal treated under carbon dioxide environment and Lyle: effects on haematology, histology, and biochemical indices in swine. *Tropical Journal of Animal Science*. 6(1): 145-154.
[8] Bass, N.H., Netsky, M.G. and Young, E. (1970). Effect of neonatal malnutrition on developing cerebrum. Microchemical and histologic study of cellular differentiation in the rat. *Arch. Neurol. (Chic)*. 23: 303-313.
[9] Britt, J.H., Armstrong, J.D. and Cox, N.M. (1988). Metabolic interfaces between nutrition and reproduction in pigs. *Proceedings of the 11[t] International Congress of Animal Reproduction and Artificial Insemination, Dublin*. 5: 117-125.
[10] Brown, J.M., and Hazen, S.L. (2015). The gut microbial endocrine organ: bacterially derived signals driving cardiometabolic disease- -s. *Annual review of medicine*, 66: 343-359.
[11] Dallman, M.F., La Fleur, S.E., Pecoraro, N.C., Gomez. F., Houshyar, H., and Akana, S.F. (2004). Minireview: Glucocorticoids-Food Intake, Abdominal Obesity, and Wealthy Nations in 2004, *Endocrinology*. 145 (6): 2633-2638.
[12] Dubey, A.K., Cameron, J.L., Steiner, R.A., and Plant, T.M. (1986). Inhibition of gonadotropin secretion in castrated male rhesus monkeys (Macaca mulatta) induced by dietary restriction: analogy with the prepubertal hiatus of gonadotropin release. *Endocrinology*. 118: 518-525.
[13] Egbunike, G.N. 1981. Regional distribution of acetylcholinesterase activity in the brain and hypophyses of crossbred European boars reared in the humid tropics. *Acta. Anat*. 110: 248-252.
[14] Ellman, G.L., Courtney, K.D., Andres, V.Jr. and Feathermore, R.M. (1961). A new and rapid colorimetric determination of acetylcho--linesterase activity. *Biochem. Pharmac*. 7: 88-95.
[15] Fonda, E.S., Rampacek, G.B. and kraeling, R.R. (1984). The effect of adrenocorticotropin or hydrocortisone on serum luteinizing hormone concentrations after adrenalectomy and/or ovariectomy in the prepubertal gilt. *Endocrinology*. 114: 268-273.
[16] Frandsen, R.D., Wilke, W.L. and Fails, A.D. (2009). Anatomy and Physiology of farm animals. 7th Ed. Wiley-Blackwell, Iowa, USA pp. 140-141.
[17] Neely, G.A., Sabir, S. and Kohli, A. (2023). Neostigmine. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing; August 8, 2023.
[18] Im, H.S., Barnes, R.H., Pond, W.G. and Levistsky, D.A. (1973). Post-natal malnutrition and regional cholinesterase activities in brain of pigs. *Brain Research*.63: 461-465.
[19] Jones, B.E. (2005). From waking to sleeping: Neuronal and chemical substrates. *Trends Pharmacol. Sci.* 26: 578–586. doi: 10.1016/j. tips.2005.09.009.
[20] Keisuke, S., Channa, N.J., and Stephen, R.B. (2011). "The Gut Hormones in Appetite Regulation", *Journal of Obesity*. Article ID 528401, 10 pages, 2011. https://doi.org/10.1155/2011/528401
[21] Kurth, F., Gaser, C. and Luders, E. (2021). Development of sex differences in the human brain. *Cognitive Neuroscience*. 12 (3-4): 155-162.
[22] Mladenović, M., Arsić, B.B., Stanković, N., Mihović, N., Ragno, R., Regan, A., Milićević, J.S., Trtić-Petrović, T.M. and Micić R. (2018). The Targeted Pesticides as Acetylcholinesterase Inhibitors: Comprehensive Cross-Organism Molecular Modelling Studies Performed to Anticipate the Pharmacology of Harmfulness to Humans In Vitro. Molecules. 23 (9): 2192. doi: 10.3390/molecules2309 2192. PMID: 30200244; PMCID: PMC6225315.
[23] McHardy, S.F., Wang, H.L., McCowen, S.V., and Valdez, M.C. (2017). Recent advances in acetylcholinesterase inhibitors and reactivators: an update on the patent literature (2012-2015). Expert Opin. Ther. Pat. 27 (4): 455-476.
[24] McKay, L.I., and Cidlowski, J.A. (2003). Physiologic and Pharmacologic Effects of Corticosteroids. In: Kufe, D.W., Pollock, R.E., Weichselbaum, R.R., *et al*. editors. Holland-Frei Cancer Medicine. 6th edition. Hamilton (ON): BC Decker; 2003. Available from: https://www.ncbi.nlm.nih.gov/books/NBK13780/
[25] Nagini, S., Palrasu, M. and Bishayee, A. (2023). Limonoids from neem (*Azadirachta indica* A. Juss.) are potential anticancer drug candidates. Med Res Rev. 2023 Aug 17. doi: 10.1002/med.21988. Epub ahead of print. PMID: 37589457.
[26] Packard, M.G., Kohlmaier, J.R., and Alexander, G.M. (1996). Post-training intrahippocampal estradiol injections enhance spatial memory in male rats: Interaction with cholinergic systems. *Behavioural Neuroscience*, 110 (3): 626-632. https://doi.org/10.1037/0735-7044.110.3.626
[27] Rajagopalan, V., Venkataraman, S., Rajendran, D.S., Kumar, V.V., Kumar, V.V. and Rangasamy, G. (2023). Acetylcholinesterase biosensors for electrochemical detection of neurotoxic pesticides and acetylcholine neurotransmitter: A literature review, Environmen--tal Research, Volume 227, 115724.
[28] Rees, T.M. and Brimijoin, S. (2003). The role of acetylcholinesterase in the pathogenesis of Alzheimer's disease. Drugs Today (Barc). 39 (1): 75-83. doi: 10.1358/dot.2003.39.1.740206. PMID: 12669110.
[29] Reis, D.J., Ross, R.A., and Joh, T.K. (1977). Changes in the activity and amount of enzymes synthesizing catecholamines and acetylcholine in brain, adrenal medulla and sympathetic ganglion of aged rat and mouse. Brain Research. 86:465-474.
[30] Ristori, J., Cocchetti, C, Romani A, Mazzoli F, Vignozzi L, Maggi M, and Fisher A.D. (2020). Brain Sex Differences Related to Gender Identity Development: Genes or Hormones? *International Journal of Molecular Sciences*. 21(6): 2123. https://doi.org/10.3390/ ijms21062123
[31] Ritzman, S.E. and Daniels, J.C. Eds. (1979). Serum protein abnormalities: diagnostic and clinical aspects. Boston: Little Brown and Company.
[32] Ryan, S., and Arnold, A.P. (1979). Evidence for cholinergic mechanisms in brain regions related to bird song. *Soc. Neuro. Sci. Abstra--ct*. 5: 146.

[33] Sawada, N., Takanaga, H., Matsuo, H., Naito, M., Tsuruo, T. and Sawada, Y. (1999). Choline Uptake by Mouse Brain Capillary Endothelial Cells in Culture, *Journal of Pharmacy and Pharmacology*, Volume 51, Issue 7, July 1999, Pages 847-852, https://doi.org/10.1211/0022357991773050
[34] Strazielle, C., Kremarik, P., Ghersi – Egea, J.F. and Lalonde, R. (1998). Regional brain variations of cytochrome oxidase activity and motor coordination in Lurcher mutant mice. Exp. Brain Resh. 121: 35 – 45.
[35] Si Shangkun, Zhao Xiaohu, Su Fan, Lu Hongxiu, Zhang Dongbin, Sun Li, Wang Fulei, and Xu Li (2023). New advances in clinical application of neostigmine: no longer focusing solely on increasing skeletal muscle strength. Frontiers in Pharmacology. VOLUME= 14. DOI=10.3389/fphar.2023.1227496
[36] Thompson, R.F. (1985). The Brain-An Introduction to Neuroscience. W.H. Freeman and Company, New York.
[37] Ure, A., Cox, G.R., Haslam, R. and Williams, K. (2023). Acetylcholinesterase inhibitors for autistic spectrum disorders. Cochrane Database Syst Rev. 2023 Jun 1;6(6):CD013851. doi: 10.1002/14651858.CD013851.pub2. PMID: 37267443; PMCID: PMC10233795.
[38] Vitorović-Todorović, M.D., and Vujatović-Velimirov, T. (2023). The reversible inhibitors of acetylcholinesterase as pretreatment options against nerve agents' intoxications. In Sensing of Deadly Toxic Chemical Warfare Agents, Nerve Agent Simulants, and their Toxicological Aspects. Chapter 21. Editor(s): Sangita Das, Sabu Thomas, Partha Pratim Das, Elsevier. Pp 503-528.
[39] Wiedeman, A.M., Barr, S.I., Green, T.J., Xu, Z., Innis, S.M. and Kitts, D.D. (2018). Dietary Choline Intake: Current State of Knowledge Across the Life Cycle. *Nutrients*. 10(10):1513. https://doi.org/10.3390/nu10101513
[40] Wurtman, R.J., Cansev, M., Ulus, I.H. (2009). Choline and Its Products Acetylcholine and Phosphatidylcholine. In: Lajtha, A., Tettamanti, G., Goracci, G. (eds) Handbook of Neurochemistry and Molecular Neurobiology. Springer, Boston, MA. https://doi.org/10.1007/978-0-387-30378-9_18.

AUTHOR'S PROFILE

First Author

Sokunbi, Olujide Adedamola, (Ph.D., RAS) Presently, an Associate Professor (Animal Physiology) in the Department of Animal Science, University of Ibadan, Nigeria. An awardee of the MacArthur Foundation Overseas Training Grant (at Purdue University, USA), a Council member (representing researchers and practitioners in the area of Animal Physiology and Bioclimatology) of the Nigerian Institute of Animal Science, and resource person, Animal Genetic Resource (AnGR) Project, National Biotechnology Development Agency (NABDA). Involved in research on nutrition and reproductive physiology of poultry, swine, rabbit, and goats; with over 100 publications in journals and referred conferences. Sokunbi--'s research focus has been in utilizing physiological and biochemical tools such as haematology, serum biochemical assays, histology, and neuroendocrinology to investigate acceptable thresholds of inclusion of anti-nutrients (most especially alkaloids) into animal feed formulation. Presently, his research focus is on the development of local semen extenders/materials for porcine, caprine and poultry.

Second Author

Jovi Richard Otite, (Ph.D Reproductive Physiology) Jovi Richard Otite currently works at Charles River Laboratories, Senneville, Quebec, Canada. Otite has over 25 years industrial and research experience in the field of animal reproductive physiology and practical experience in animal cloning, ICSI and in vitro fertilisation. He is a pioneer researcher in the field of companion Animal Science in Nigeria with a textbook on reproduction in the dog, a tropical approach. Otite is currently involved in preclinical research involving the use of genetic engineered animals such as disease germ free and immunodeficient animal models in bid of evaluating potential cures for both human and animal diseases. He has worked -on a wide range of animal species such as Dogs, Cats, Snakes, Monkeys (African Green and Rhesus) as well as Cattle, Pigs, Pangolins and Parrots. Jovi's future research lies in the production of endangered species through trans-specie cloning.

Third Author

Abiola John Olusoji, (DVM, MVSC, FCVSN) Lecturer at the Department of Veterinary Medicine with specialization in Animal Husbandry and Management. Has over 12 years of teaching and research in animal husbandry and management at the higher institutions. Abiola has a special interest in animal behaviour and welfare especially on the improvement of the animal welfare in the country. He is a member of Animal Welfare Club in University of Ibadan with members across various faculties. He is also staff adviser to students' farm research foundation in UI, a body that believes in practical involvement of students in Animal Husbandry. He is a consultant to different livestock farms in the South West of Niger--ia.

Fourth Author

Olufemi Alaba, (Ph.D., RAS) Presently a Senior Lecturer in Animal Physiology and Bioclimatology, Department of Animal Science, Faculty of Agriculture at the University of Ibadan with over 12 years of professional experience in teaching and practice of Animal Science. His area of research includes reproductive, nutritional, environmental physiology as well as neurophysiology. He is the current postgraduate coordinator in the Department of Animal Science. He has published some scientific articles in international and local journals, most of them reporting original research results.