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Full Length Research Paper

Brain Development and Regional Histomorphometry of Growing Male Pigs Fed Varying Levels of *Spondiasmombin* Leaf Meal

Alaba O, Muraina HA and Ogunwumiju B

Animal Physiology and Bioclimatology Unit, Department of Animal Sciences, University of Ibadan, Ibadan, Nigeria

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This study aimed at evaluating some metabolite characteristics of the growing pig brain, morphometry and response to the dietary composition of *Spondiasmombin* leaf meal (SMLM).Twelve (12) growing male pigs weighing 13kg were randomly allotted to four treatment-supplemented maize feed ingredients with T1 to T4: 0, 5, 6 and 7% with *Spondiasmombin* leaf meal (SMLM) w/w in a completely randomized design with three animals per treatment and one animal in each replicate. Glucose and total protein concentrations in both blood and brain were assessed as well as morphometry of different brain regions; cerebrum alongside hemispheric difference, cerebellum, hypothalamus, pons, hippocampus, amygdala and pituitary. Results show that r^2 of 0.46 shows that 46% of the weight observed is due to the feed offered and means of glucose concentration in the brain significantly differ among treatments with T4 as the highest. also, higher blood-brain protein concentrations in T2 and T4were similar but significantly different from others. The same level of protein in blood and brain was observed in T3 which indicates little is available for tissue formation and weight gain. This study shows that SMLM inclusion in diets of up to 7%, increase in crude protein and development of different brain regions were observed in glucose and total protein concentration in the brain of animals.

Keywords: Nutrition, response, Spondias Mombin Leaf Meal, brain, gluconeogenetic, protein

INTRODUCTION

Globally, there is an increasing population of pigs in developed countries like the United States of America where more than ten million pigs are slaughtered each year (Machete and Chabo, 2020; Oyebanjo, *et al*., 2023).Pig production is an activity that is practiced widely across Africa and has been contributing immensely to the agricultural economy of the continent, especially, as a means of livelihood for many of her rural population (Adesehinwa *et al*.,2024).The Swine industry has a major economic impact on agriculture in Nigeria; it is an important arm of the Nigeria livestock sub-sector in the overall agricultural sector (Ezeibe, 2010). It plays an by important role in the livelihood of livestock farmers by providing economic, social and food security. Nutrition is a key factor that affects the growth and development of an organism and it has an intense effect on the physiological responses of vital organs in the body of animals. Due to the increase in demand for food sources and they rise in population, the quest for alternative feed ingredients has been of keen interest to farmers (Baumgard and Rhoads, 2013). Feed quality is particularly a major factor that ensures the success or otherwise of pig production (Okunnade *et al*., 2010). One of Nigeria's main obstacles to livestock improvement is inadequate and unbalanced feed. This development has immensely affected livestock production level, particularly pigs (Oluyemi and Roberts, 2000). Although food has classically been perceived as a means to provide energy and building material to the body, the relative abundance of specific nutrients can affect cognitive processes and

Corresponding author's Email: femialaba@gmail.com, +2347054634435

emotions. In particular, research in recent times has provided exciting evidence for the influence of dietary factors on specific molecular systems and mechanisms that maintain mental function. Newly described influences of dietary factors on neuronal function and synaptic plasticity have revealed some of the vital mechanisms responsible for diet's action on brain health and mental function (Gómez-Pinilla, 2008).Nutrients gained from diets influence specific molecular systems and mechanisms that support the cognitive and mental function of the brain. However, there is a need to evaluate the influence of feedstuffs and inherent anti-nutritional factors on brain development. The *Spondiasmombin* is called Hog plum in English, *akika* in Yoruba, *ijikara* in Igbo, *tsader maser* in Hausa, *chabbuli*in Fulani and *nsukakara*in Efik (Gill, 1992). *S. mombin* leaves are among the forages given to domestic animals in South-Eastern Nigeria. The young leaves are also cooked as green vegetables (Ayoka *et al*, 2008). This study aims at the enhancement of swine production and reducing the cost of feeding by the use of cheap feedstuff through the use of *Spondiasmombin* leaf meal to supply essential nutrients necessary for growth.

MATERIALS AND METHODS

Experimental location

The study was carried out at the Teaching and Research Farm (TRF) of the University of Ibadan, Ibadan located between latitudes 60°10" and 90°10" north of the equator and longitudes 30° and 60° of the Greenwich during the hot dry season (February to March 2020). Twelve (12) growing male pigs weighing 13kg were randomly allotted to four treatments supplemented maize feed ingredient with T1 to T4: 0, 5, 6 and 7% with *Spondiasmombin* leaf meal (SMLM) w/w in a completely randomized design with three animals per treatment with one animal in each replicate. At the end of the feeding trials the pigs were sacrificed, heads dissected to harvest the brain, weighed and separated into regions samples were taken from the following regions: amygdala, cerebellum, cerebrum, hypothalamus, hippocampus, pons varoli, mid-brain and medulla oblongata.

Table 1: Percentage Composition of Experimental Diet (DM- Basis)

Ingredient	T1	T2	T3	T4
Maize	27	22	21	20
SMLM	0	5	6	7
Palm Oil	3	3	3	3
SBM	17	17	17	17
Wheat Offal	22	22	22	22
PKC	25	25	25	25
Fish Meal	1	1	1	1
DCP	2.5	2.5	2.5	2.5
Oyster shell	1.50	1.50	1.50	1.50
Premix	0.25	0.25	0.25	0.25
2Salt	0.25	0.25	0.25	0.25
Lysine	0.30	0.30	0.30	0.30
Methionine	0.20	0.20	0.20	0.20
Total	**100.00**	**100.00**	**100.00**	**100.0**
Calculated analysis				
ME (kcal/kg)	2527.4	2512.73	2509.72	2506.71
Crude Protein (%)	17.00	18.02	18.28	8.54
Crude Fibre (%)	7.5	8.22	18.36	8.50

***DM =Dry Matter**

Glucose concentration

The glucose concentrations in the regions of the brain were evaluated using the method earlier reported by Bitto and Gemade (2001). 10 mL of the homogenate was introduced into a test tube and l mL of the glucose reagent was added. The mixture was incubated at 37°C for 10 min. Some of the incubated mixture was poured into a clean cuvette and read at a wavelength of 500 nm.
Glucose concentration (mg/dL) = Absorbance of homogenate × 100 / Absorbance of standard

Total proteins concentration

The total protein concentrations in the regions were evaluated using the Biuret method as earlier reported by Adejumo and Egbunike (2001). An automatic dispenser was used to measure 5 mL of Biuret reagent into a test tube and 10 mL of the homogenate was also added. The mixture was incubated at room temperature of about 25ºC for 30 min. After incubation, the incubated mixture was poured into a clean cuvette. The side of the cuvette was thoroughly wiped with tissue paper before it was placed inside the spectrophotometer at a wavelength of 540 nm to determine the protein concentration. The blank was used to standardize the spectrophotometer. The standard was prepared using 0.1 mL of total protein standard and 5 mL of Biuret reagent.
Total protein concentration = Absorbance of homogenate.

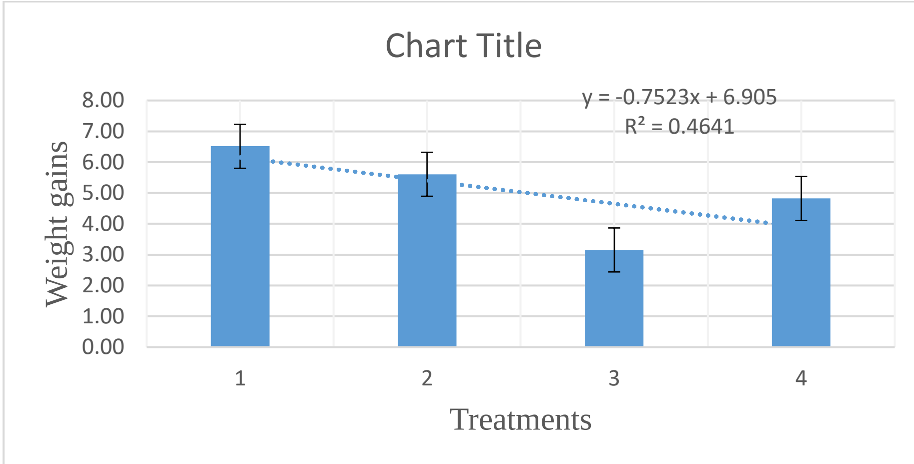

Figure 1: Average weight gain performance of each treatment with varying inclusion levels of SMLM

Table 2: Glucose and total protein concentration in blood and brain of pigs fed SMLM

Treatments					
Parameters	T1	T2	T3	T4	SEM
TP_a(g/dL)	6.25^{ab}	5.63^{b}	6.45^{ab}	7.19^{a}	0.19
TP_b (g/dL)	6.09	7.46	6.45	7.56	0.25
TP_b:TP_a	0.97^{b}	1.32^{a}	1.00^{b}	1.05^{ab}	0.05
Glu_a (mg/dL)	58.42	49.39	48.91	58.60	1.87
Glu_b(mg/dL)	29.15^{b}	53.91^{ab}	64.60^{ab}	81.27^{a}	0.97
Glu_b :Glu_a	0.49^{b}	1.08^{ab}	1.31^{a}	1.38^{a}	0.11

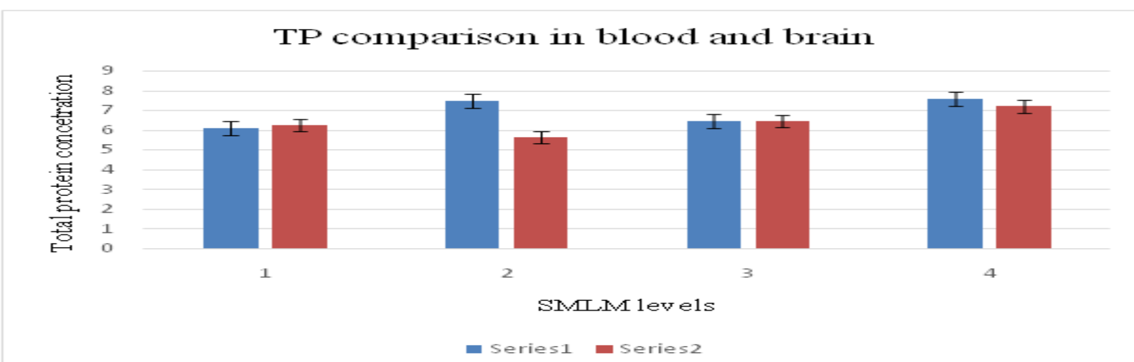

Figure 2: Comparison of total protein concentration in blood and brain of pigs fed different levels of SMLM
The blue bar shows TP in the blood while the brown shows TP concentration in the brain

6 g/dL / Absorbance of standard.
There is a significant difference in total protein concentration in the blood of experimental animals, which may be attributed to the high protein content of SMLM. T4 which is the highest level of SMLM inclusion of 7% shows highest TP concentration. Also, the means of glucose concentration in the brain significantly differ among treatments with T4 as the highest. The means of average values in TP concentration in the brain and glucose in the blood are similar. Ratios of blood: Brain total protein and glucose concentrations significantly differ across treatments with the highest values obtained in T4.TP_a: Total protein concentration in blood, TP_b: Total protein concentration in brain, Glu_a: Glucose concentration in blood, Glu_b: Glucose concentration in brain.

Graph showing a comparison between total protein concentration in blood and brain of pigs fed different levels of SMLM

Figure 2 shows a comparison of total protein concentration in the blood tissue and brain tissue of pigs. The highest difference can be observed in T2, while other

Table 3: Morphometry of different brain regions of pigs fed varying levels of SMLM

	Treatments				
Parameters	**T1**	**T2**	**T3**	**T4**	**SEM**
Brain weight (g)	70.07	83.29	84.60	82.44	5.99
Pituitary (g)	0.72	0.04	0.09	0.01	0.08
Hypothalamus (g)	0.08	0.12	0.14	0.12	0.01
Cerebellum (g)	8.67	7.26	5.88	6.16	0.59
Rt. C. hemisphere	30.48	30.41	27.33	34.69	1.60
Lt. C. hemisphere	24.25	25.42	24.81	29.29	0.76
Amygdala (g)	0.23	0.13	1.55	1.13	0.18
Hippocampus (g)	0.31	0.11	0.49	0.42	0.05
Pons (g)	0.16	0.21	3.30	2.41	0.42

treatment averages are comparable while T3 has the same concentration in blood and brain.

Histomorphometry of growing pigs' brains fed different levels of SMLM

The weights of the whole brain and different brain regions are shown in Table2. Animal in T3 shows the average highest weights compared to other treatments, although do not significantly differ in comparison.

DISCUSSION

Growth performance and feed efficiency

Nutritional level is one of the factors which affect the growth and physiology of animals. Animals on treatments 1 and 2 show better and similar growth responses as a nutrient level of each diet used in the experiment supplies adequate quantity and proportion needed for development. This experiment with an r^2 of 0.46 shows that 46% of the weight observed is due to the feed offered. Other factors like genetic purity and expression, aggressiveness etc may affect the results shown in the graph with pigs in T3 having the least, though not significant, weight gain. This is reflected in their feed conversion efficiencies. Rauw *et al.,* (2017) posited that animals that grow faster with higher FCE, are phenotypically independent of body weight gain but highly correlated with feed intake. These features can be affected by various stress exposures from dominance behaviour, and pecking order to heat tolerance. Maize ingredients supplemented with SMLM in diets with up to 7% do not have adverse effects on the growth and performance of growing pigs and contain adequate chemical composition value to meet the nutrient requirement of animals.

Blood-brain metabolites concentration

Glucose

It is a primary energy source and the main carbon source for fatty acid synthesis in most living organisms including mammals. The continual supply of glucose is important for various physiological processes as well as nervous body mechanism coordination. To maintain continuous supply, the gluconeogenetic pathway meets the need of the body for glucose when main energy sources are not available or in sufficient amounts from the diet. Means of glucose concentration in the brain significantly differ among treatments with T4 as the highest. This is due to the considerable contribution of SMLM to the energy/soluble starch level of diet and a veritable source of gluconeogenetic substrate high in crude protein and fibre. Starch digestion kinetics is a rising issue in pig nutrition (Menoyo *et al*., 2011), as well as important in poultry nutrition due to their implication for productive performance, nutrient digestibility, amino acids metabolism feed consumption (Alagawany *et al*., 2021),carcass composition and physiological responses (Giuberti *et al*., 2012). Some other factors also affect the rate and degree of starch digestion, they include; age, physiological status, feed consumption, passage rate and absorptive capacity of the gastrointestinal tract.

Different studies have been reported on the effect of different glucose sources with various physiochemical properties on post-prandial blood glucose and physiological response in swine Gallo and Masoero, 2012; Jarrett *et al*., 2018; Pietro *et al*., 2020; Eliana *et al*., 2022 on the assumption that the final stage of glucose production. There is no significant difference in blood glucose concentration across all treatments. However, glucose concentrations in T1 to T4 significantly differ with the highest concentration in T4 and lowest in T1 with 0% SMLM inclusion. *Spondiasmombin* leaf meal is high in metabolizable energy, 2650Kcal/kg (Okonkwo and Esiegwu, 2018), The glucose level in cerebrospinal fluid, CSF is proportional to the blood glucose level and corresponds to 60-70% of the concentration in blood (Mundt and Shanahan, 2010). Therefore, normal CSF glucose levels lie between 2.5 and 4.4 mmol/L (45–80 mg/dL). All mean values from T1 to T4 fall within this range. There is no pathologic process that directly leads to high CSF glucose levels and therefore, high CSF glucose levels have no specific diagnostic importance. However, elevated blood sugar levels (hyperglycemia) result in elevated CSF glucose levels (Seehusen *et al*., 2003) as the CSF glucose level is proportional to the blood glucose level with glucose being actively transported as well as simply diffusing down the concentration gradient from blood to CSF.

Total Protein

The nutritional status of an animal has a marked effect on the synthesis of plasma proteins. Lack of dietary protein (invariably impaired protein availability) has its most marked effect on the levels of gamma globulins and albumin. In this study, animal diets with 7% SMLM inclusion show the highest concentration of total protein in T4 both in blood and brain (Mathew *et al*., 2022) stated that plasma proteins serve as a source of nutrition for tissues and dynamic equilibrium exists between proteins of plasma and those of tissue. These positions support the marked increase in growth and weight gain of pigs in these groups. The study of proteins in cerebrospinal fluid (CSF) is of importance to establish blood-brain barrier damage and the synthesis of immunoglobulins (Ig) within the central nervous system (CNS). Figure 2 shows higher blood-brain protein concentrations in T2 and T4 animals which are similar but significantly differ from others. The same level of protein in blood and brain is observed in T3 which indicates little is available for tissue formation and weight gain. Kadry *et al*., (2020) posited that having more protein in the brain than blood may indicate signs of infection or damage to the blood-brain barrier and subsequently brain tissues. Ewuola and Bolarinwa 2017; Uchechukwu *et al*., 2024 posited that Protein in the brain functions for repair of worn-out tissues, growth, muscular development, and also binds to some minerals to ensure their bioavailability and proper utilization as found in SMLM used in the present study.

Histomorphometry of growing pigs brain regions fed different levels of SMLM

The weights of the entire brain and the various brain regions are displayed in Table 3. Though they are not statistically different, animals in T3 exhibit the average highest weight when compared to other treatments. This further explains the even distribution of total protein concentration from diet in blood to brain as shown in figure 2 leading to more synthesis of neurons and greater neural activities in animals which could be as a result or reaction of any cause.

The pituitary gland is called the “master gland” because its hormones regulate other important endocrine glands—including the adrenal, thyroid, and reproductive glands and in some cases have direct regulatory effects in major tissues, such as those of the musculoskeletal system. Although similar to other treatments, higher pituitary weight shown in T3 than in T1 and T4, indicates more hormonal activities and coordination needs both neural and developmental. The same trend is observed in weights of the hypothalamus, hippocampus, amygdala and pons. However, the scenario is reversed as observed in cerebral and cerebella weights.

It is fascinating to note that the weight of the right cerebral hemisphere was higher than that of the left hemisphere. Cerebral asymmetry has been noticed in the brains of several species and this has been implied by scientists to have behavioural and cognitive effects like learning patterns and emotional display (Camerlink, *et al.,*2018). Rentería (2012) perceived the prominent role that cerebral asymmetry plays in the organization of the brain, and its possible implication in neuro developmental and psychiatric conditions.

CONCLUSION

Nutrition is a key factor that affects the growth and development of an organism. Nutrition has an intense effect on the physiological responses of vital organs in the body of animals. Due to an increase in demand for food sources due to an increase in population, the quest for alternative feed ingredients has been of keen interest to farmers. As observed in this study, the inclusion of *Spondaismombin* leaf meal (SMLM) has a significant effect on the growth rate and performance of growing male pigs, reducing the high cost of energy and protein source ingredients in feed formulation and promoting animal physiological activities. In addition to this study, as SMLM inclusion in diets up to 7%, an increase in crude protein and development of different brain regions were observed in glucose and total protein concentration in the brains of animals.

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