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Weight Variation and Its Effect on Acetylcholinesterase Activity and Total Protein Concentration in the Brain Regions and Hypophyses of Pigs in the Tropics

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Abstract: In an experiment to determine the effects of variations in weight on the acetylcholinesterase (AChE) activity and total protein concentration in the brain regions and hypophyses of pigs commercially reared in the tropics, a total of 80 pigs were sorted into 8 body weight categories with 10 pigs in each body weight category. The pigs were sacrificed and their brain regions and hypophyses assayed for AChE activity and for total protein concentration. The body weight categories were 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, 81-90 and 91-100kg. Both sexes were equally represented in each category. Acetylcholinesterase activity was highest in the amygdala, hippocampus and mid-brain of pigs within the 41-70kg weight categories and lowest in the cerebral cortex of pigs within the 31-40kg weight category although the differences were not significant ($P>0.05$). Variations in mean Specific Acetylcholinesterase (SACHE) activity and in mean concentration of total proteins with pig body weight and brain regions/hypophyses were highly significant ($P<0.01$). Specific Acetylcholinesterase (SACHE) activity was highest in amygdala, hippocampus and hypothalamus of pigs within the 41-70kg weight categories and lowest in the cerebellum and cerebral cortex of pigs within the 31-40 kg weight category. Total protein concentration was highest in the pons and cerebellum of pigs within the 31-40 and 91-100kg weight categories. Total protein concentration was lowest in the hypophyses of pigs within the 41-50kg weight category however; there were no observed variations in relation to AChE activity. It was concluded that such factors such as differences in

age, nutrition and management of the pigs may have informed the non-specific variation patterns of the variables observed in this study.

Keywords: Pigs, acetylcholinesterase, body weights, brain, hypophyses, amygdala, hippocampus, hypothalamus, pons, medulla oblongata

INTRODUCTION

Food security, especially of animal protein origin, still poses great challenges to many countries especially in tropical Africa. In a country such as Nigeria with a large human population and an under-developed livestock subsector, a wide gap still exists between demand and supply of livestock and poultry products. Consequently meat, milk and eggs that are in short supply, command high prices and remain out of reach of majority of the populace¹.

The pig, though discriminated against on religious grounds in some areas of Nigeria, is accepted and widely reared in some other parts, especially the south eastern and south-south areas of the country. In order to ensure maximum productivity in farm animals including pigs, the animals should be properly managed and attention should also be paid to the various physiological and metabolic activities in the body to ensure that these processes proceed optimally. By virtue of its prime position in the central nervous system, the brain plays a modulating role in all the body metabolic and physiological processes and through its control on the hypophyseal hormonal pathways, keeps the levels of hormonal production and metabolism of other glands at a steady state².

It is well documented that the brain's neurotransmitters, particularly those of the cholinergic pathways, are of vital importance to the physiological integrity and consequently, productivity of the animal^{3,4}. These neurotransmitters are selectively concentrated in different regions of the brain depending on the functions of the region^{5,6}. The activities of these neurotransmitters are usually under the control of several enzymes and multi-enzyme systems⁷. One of such enzymes is acetylcholinesterase (AChE; EC 3.1.1.7) which is an enzyme that participates in cholinergic neurotransmission. It breaks down acetylcholine which terminates the neurotransmission process⁸.

This study was carried out to determine the concentration and distribution of Acetylcholinesterase (AChE) total protein and the specific AChE activity in the brain regions and hypophyses of pigs and to ascertain if there is a change in the activities and concentration of this enzyme with variation in the body weight of the pigs.

MATERIALS AND METHODS

Eighty (80) Yorkshire pigs were slaughtered and their brains removed for the study. The study was conducted in Ibadan (7.38° N and 3.93°E) in the Southwestern region of Nigeria. The pigs were slaughtered in line with internationally accepted standards. Before slaughtering, the pigs were randomly selected, weighed and separated into the following eight body weight categories, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, 81-90, 91-100kg, with 10 pigs in each weight category. Both sexes were equally represented. Immediately after slaughter, the pigs were decapitated; the heads were quickly sawn open and samples taken from the hypothalamus, cerebellum, pons, cerebral cortex, amygdala, hippocampus, mid-brain and medulla oblongata as described by Egbunike⁹. The hypophyses were also removed and each was carefully dissected into the adeno-hypophysis and neuro-hypophysis. Each brain or hypophyseal sample was then homogenized in a 0.1molar ice-cold phosphate buffer (pH 7.4; 1% w v⁻¹) using a Potter-Elvehjem glass homogenizer and immediately

assayed for AChE activity and total protein content. AChE activity was determined according to the colorimetric method described by Egbunike⁹, which measures the rate of hydrolysis of thiocholine and acetate using 5:5 dithiobis-2-nitrobenzoate (DTNB) as the color reagent. AChE activity was expressed in mmole g⁻¹ wet tissue min⁻¹. Total protein was assayed by the modified biuretic method as described by Egbunike⁹ and expressed in g 100ml⁻¹.

All data collected were subjected to a two way analysis of variance in a complete randomized block design. Means were separated using Duncan's Multiple Range Test¹⁰.

RESULTS AND DISCUSSION

Results of the effects of body weight variation and brain/hypophyseal region on AChE activity and total protein concentration are presented in Table 1. Variations in mean AChE activity were not significant ($P>0.05$), however highly significant ($P<0.01$) differences in AChE activity were observed in the different brain regions and hypophyses of the experimental animals. Highly significant ($P<0.01$) differences were observed in total protein concentration with variation in body weights. Significant ($P<0.01$) differences in total protein concentrations were also observed in the different brain regions/hypophyses of the experimental animals.

The mean AChE, specific AChE activities and total protein concentration of the experimental animals within the different weight categories and in the brain regions/hypophyses are presented in Tables 2 and 3. Pigs within 41-70kg weight categories had the highest mean AChE values (3.797-4.250 mmole g⁻¹ wet tissue min⁻¹). Higher SACHe values were also observed for pigs within the 41-80kg weight categories (25.009-32.241 mmole g⁻¹ wet tissue min⁻¹). Pigs within the 31-40 and 91-100kg weight categories had lower values for SACHe activity (5.993-8.599 mmole g⁻¹ wet tissue min⁻¹). Total protein concentration was significantly ($P<0.01$) higher in the 31-40 and 91-100kg categories than in the other body weight categories. Pigs within the 41-50kg category had the lowest mean total protein concentration.

The amygdala was the most active region of the brain in terms of AChE and SACHe activity (5.563 and 40.392 mmole g⁻¹ wet tissue min⁻¹ respectively). The cerebral cortex had the lowest AChE activity while the cerebellum recorded the lowest SACHe activity. Mean total protein concentration was highest in the pons and cerebellum (0.528 and 0.514g 100ml⁻¹ respectively) and lowest in the hypophyses. These results demonstrate the existence of a body-weight related differential distribution of total protein and AChE activity in the brain and hypophyses of the pig. Generally, AChE activity was highest in the amygdala, hippocampus and mid-brain (mesencephalon) of the experimental animals within the body weight categories of 41-70kg and least in the cerebral cortex of pigs within the 31-40kg body weight category.

These regional differences are in agreement with the findings of Moudgil and Kanungo¹¹, Egbunike⁹, Adejumo and Egbunike^{12,13}. Although low AChE activity was generally accompanied by low SACHe activity and vice-versa as observed by Adejumo and Egbunike¹², total protein concentration did not vary in the same proportion. Other factors such as age of animal, nutritional status and environmental factors to which the animals may have been exposed, which are known to influence cholinergic activities in pigs² but which could not be kept constant in this study, may have accounted for this disparity.

Table- 1: Analysis of variance on variation of AChE activity and total protein concentration with body weight and brain region/hypophyses of the experimental animals.

Mean squares				
Source of variation	Degree of freedom	AChE	SChE	Total
Total	79	2.197 ^{ns}	997.702 ^{**}	0.140 ^{**}
Body Weight/brain region	7	18.230 ^{**}	821.479 ^{**}	0.122 ^{**}
Hypophyses	9	2.195	217.637	0.033
Residual	63			

AChE = acetylcholinesterase; SChE= specific acetylcholinesterase; ns = not significant ($P>0.05$); ** highly significant ($P<0.01$).

Table- 2: Variations in AChE activity and total protein concentration with body weight variation of the experimental animals

Parameters	Body weight categories (kg)								SEM
	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100	
AChE [*]	3.100 ^c	2.745 ^c	3.797 ^a	3.866 ^a	4.250 ^a	3.360 ^{bc}	3.753 ^b	3.617 ^b	0.168
SChE [*]	18.336 ^c	5.993 ^d	32.241 ^a	30.063 ^a	25.099 ^b	28.092 ^{ab}	16.070 ^c	8.599 ^d	3.493
Total Protein ^{**}	0.285 ^{bc}	0.516 ^a	0.208 ^c	0.365 ^b	0.315 ^{bc}	0.288 ^{bc}	0.304 ^{bc}	0.493 ^a	0.038

AChE = acetylcholinesterase; SChE = specific acetylcholinesterase; * $\text{mmole g}^{-1} \text{ wet tissue min}^{-1}$; ** g 100ml^{-1} ; ^{abc} Means with different superscript within rows differ significantly ($P<0.01$)

Table- 3: Variation in AChE activity and total protein concentration with brain regions/hypophyses of the experimental animals

Para-meters	Brain region/hypophyses										SEM
	pons	cb	amg	hipocam	hipothal	cer.cor	mb	med.obl	adeno-	neuro-	
AChE [*]	3.30 ^d	3.53 ^d	5.56 ^a	4.71 ^b	4.46 ^{bc}	1.91 ^c	4.89 ^b	4.13 ^c	0.76 ^f	2.36 ^e	0.477
SChE	12.49 ^{cd}	9.35 ^d	40.39 ^a	27.02 ^b	26.34 ^b	10.23 ^{cd}	23.19 ^b	15.59 ^c	12.55 ^{cd}	28.46 ^b	3.21
Total prot. ^{**}	0.53 ^a	0.52 ^a	0.35 ^{bc}	0.29 ^{cd}	0.36 ^{bc}	0.29 ^{cd}	0.26 ^{cd}	0.47 ^{ab}	0.19 ^d	0.19 ^d	0.039

AChE=acetylcholinesterase; SChE = specific acetylcholinesterase; * $\text{mmole g}^{-1} \text{ wet tissue min}^{-1}$; ** g 100ml^{-1} , cb=cerebellum; amg=amygdala, hipocam=hipocampus; hipothal=hypothalamus; cer.cor= cerebral cortex; mb=mid-brain; med.obl= medulla oblongata; adeno- = adeno-hypophyses; neuro- = neuro-hypophyses.

^{abcd} Means with different superscripts within rows differ significantly ($P<0.01$).

CONCLUSION

Significant variations in acetylcholinesterase activity and in protein concentrations with body weights and in different brain regions of tropical pigs have been established. However, the variations followed no specific pattern, probably because of the influence of other factors such as differences in age, nutrition and management of pigs, which could not be kept constant in this study.

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