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https://dx.doi.org/10.4314/nvj.v46i1.2 **ORIGINAL ARTICLE**

Serum Activities of Some Liver Enzymes and Metabolic Responses of Apparently Healthy Large White Sows During Gestation and After Parturition.

Nweze E. C[1], Obidike R. I[2], Udem S. C[2], Uchendu C. N[2].

[1]Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria; [2]Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria. *Correspondence: Email: ernest.nweze@yahoo.com; Tel: +2348066336466

ABSTRACT

This study evaluated the effects of pregnancy and lactation on the serum liver function markers and protein profiles of large white (LW) sows. Thirty (30) sexually matured LW sows and eight (8) boars, of mean weight 45.38±1.29 kg, aged between 8 and 10 months were used for this study. The sows were randomly assigned to five groups (A to E) of six sows per group in a completely randomized design (CRD). Group A: Non pregnant (control), Group B: Early gestation (38 days), Group C: Mid gestation (78 days), Group D: Late gestation (112 days) and Group E: Post gestation (14 days postpartum). Blood samples were collected from the non-pregnant control and during four stages of gestation (early gestation, mid gestation, late gestation and post-gestation). The mean Alanine Aminotransferase (ALT) activity of group B sows was significantly ($p < 0.05$) lower than group C but showed no significant ($p > 0.05$) variations when compared to groups D, E and control. The mean Alkaline Phosphatase (ALP) activity of the treated groups (D and E) was significantly ($p < 0.05$) decreased when compared to the control. The mean total protein (TP) and globulin (G) concentrations of treated groups (B, C and D) were significantly ($p < 0.05$) higher than the control. The mean albumin/globulin (A/G) ratio of group B was significantly ($p < 0.05$) lower than the control. There were however, no significant variations ($p > 0.05$) in the mean Aspartate Aminotransferase (AST) activity, Aspartate Aminotransferase/Alanine Aminotransferase (AST/ALT) ratio and albumin concentrations among all the groups. This present study has presented preliminary information on the changes in the serum liver function and protein parameters during gestation and after parturition in LW sows.

Key words: Gestation, large white pigs, liver function, proteins, serum.

INTRODUCTION

Pig reproduction remains one of the veritable sources of supply of animal protein (Apata and Ojo, 2000; Udofia *et al.*, 2007). Reproduction of pigs provides man with a more uniform supply of meat (pork) (Chandy, 2004; Ayalew *et al.*, 2011) and by-products such as pigskin and bristle used in

the manufacture of light leather and brushes especially in Asian countries (Young, 2005). The pig's output; yield of meat/tonne of live weight of breeding pigs especially number of sows per year is about six times that of cattle (Mpofu and Makuza, 2003).

Blood serum biochemical profile testing is a pre-symptomatic diagnostic tool to assess the swine herd's nutritional status and other productive and reproductive disorders (Pathan *et al.,* 2011). The profile may vary according to factors such as origin, climate, management practices, geographical distribution, season and reproductive stage of animals (Cozzi *et al.,* 2011; Pal and Acharya, 2013; Mahima *et al.,* 2013). So, it is important to determine the blood biochemical profile for the clinical interpretation of laboratory findings especially in the post parturient stages at which the sows are more likely to suffer from metabolic disorders.

Physiological changes in pregnancy are primarily due to the changes in the hormonal concentrations (Chandra *et al.*, 2012). Pacheco *et al.* (2016) and Souza *et al.* (2018) emphasized the physiological changes related to nutrient expenditure with foetal growth, placental functioning, increased foetal covering and fluid, uterine wall and mammary glands, highlighting the increase in the metabolic rate that occurs during gestation due to the higher requirement of energy and proteins. Some studies have been developed to characterize the dynamics of biomarkers in Santa Inês (Araújo *et al.*, 2014), Dorper sheep (Soares *et al.,* 2014), Morada Nova sheep (Santos *et al.*, 2014) and Saanen and Alpine Brown dairy goats (Oliveira *et al.,* 2019) during the transition period. These studies demonstrated that the gestational period influences the metabolic response of several biochemical parameters, besides which the data serve as tools for the diagnosis of metabolic disorders that occur in the transition period. Currently, there are no data available on the serum biochemical changes that occur during the transition period in LW sows. Therefore, the aim of this present study was to determine the effects of pregnancy and lactation (reproductive stages) on the serum liver function markers and protein profiles of LW sows kept in this eco-zone.

MATERIALS AND METHODS

Experimental Animals

This experiment was carried out on LW sows obtained from Captain commercial breeding farm located in Amorji Nike, Enugu East LGA, Enugu State, Nigeria and kept in the Veterinary Teaching and Research Farm, Michael Okpara University of Agriculture, Umudike (MOUAU). Thirty (30) sexually matured cycling apparently healthy Nigerian LW sows and eight (8) boars (38 LW pigs), of mean weight 45.38±1.29 kg, aged between 8 and 10 months were used for this study. The sows and boars were acclimatized for three weeks during which they were dewormed using ivermectin at a dose of 300 µg/kg subcutaneous (SC) first, for internal and external parasites and repeated 10-14 days for second batch of maturing parasites. The pigs were kept in well-ventilated pens at room temperature (25-27^{0}C) and 12 hours light/darkness cycle maintained. Ethical approval for this study was obtained from the Research Ethical Committee of Michael Okpara University of Agriculture Teaching Hospital with ethical approval number; MOUAU/CVM/REC/202114. The animals were managed as prescribed in the Guide for the Care and Use of Large Animals of National Research Council (NRC, 1996).

Experimental Design

Thirty (30) open LW sows were randomly assigned to five groups (A to E) of six sows per group in a completely randomized design (CRD). Group A: Non pregnant (control), Group B: Early gestation (38 days), Group C: Mid gestation (78 days), Group D: Late gestation (112 days) and Group E: Post gestation (14 days post-partum). The sows were synchronized for estrus using $PGF_{2\alpha}$ given twice, 11 days apart (Akusu and Egbunike, 1984) at a dose of 10mg/kg intramuscular (IM). Two boars each were introduced to naturally serve the sows in groups B, C, D and E (pregnant groups). Following successful mating, pregnancy was confirmed by ultrasonography (B-Ultrasound scanner, Korea) (Ali and Fahmy, 2008) between days 22-23. The pregnant sows were identified with tag letters thus, B_{1-6}, C_{1-6}, D_{1-6}, E_{1-6} and kept in separate pens until farrowing and are maintained in these separate pens throughout lactation. The lactating sows (group E) after farrowing were kept in separate pens and identified thus; E_{1-6} while the non-pregnant control sows were identified thus A_{1-6} and kept together in a pen. Sows and boars were fed twice daily while the piglets received udder milk only from their dams until the end of lactation (28days). Sows, boars and piglets were provided with clean fresh water *ad libitum* throughout the period of the study. Four milliliters (4ml) of blood was collected from each sow in a group from the femoral vein and dispensed into plain test tubes and allowed to clot in a slanting position for 2 hours and centrifuged at 2,500 revolutions per minute (RPM) for 10minutes. The resulting sera were aspirated, stored at -20^0C and used to determine AST concentration (Clin. Chem. Acta 105, 1980), ALT concentration (Moss and Henderson, 1999), ALP concentration (Moss and Henderson, 1999), total protein concentration (Johnson *et al.,* 1999) and albumin concentration (Johnson *et al.*, 1999). Serum globulin concentration was derived by subtracting the value of serum albumin from the total serum protein (Cheesbrough, 2009).

Serum Globulin (g/dl) = Total serum protein (g/dl) - serum albumin (g/dl). Aspartate aminotransferase (AST)/alanine aminotransferase (AST/ALT) and albumin/globulin (A/G) ratios were calculated.

Data Analysis

Data obtained during the study were subjected to One Way Analysis of Variance (ANOVA) using statistical package for social sciences (SPSS) version 20.0. Variant means were separated using Duncan's New Multiple Range Tests (Steel and Torrie, 1986; SAS, 2010). Probability values < 0.05 were considered significant.

RESULTS

The mean ALT activity of group B sows (early gestation or first trimester) was significantly ($p < 0.05$) decreased when compared to group C but showed no significant ($p > 0.05$) variations when compared to groups D, E and the control. The mean ALP activity of the treated groups (D and E) was significantly ($p < 0.05$) decreased when compared to the control. The mean total protein (figure 4) and globulin values (figure 2) of treated groups (B, C and D) were significantly ($p < 0.05$) higher when compared to the control. The mean A/G value (figure 1) of group B was significantly ($p < 0.05$) lower when compared to the control but showed no significant ($p > 0.05$) difference when compared to groups C, D and E. There were however, no significant ($p > 0.05$) variations in the mean AST activity, AST/ALT ratio and albumin (figure 3) concentrations among all the groups.

Table 1: The mean of some serum liver enzymes of sows at different stages of gestation Values are expressed as means ± SEM in the table

Parameters	Pre-gestation (Non-pregnant control)	Early gestation (Ist trimester)	Mid gestation (2nd trimester)	Late gestation (3rd trimester)	Post-gestation (lactation)
AST (iu/l)	57.89±6.80	56.89±4.21	63.96±5.93	61.16±4.82	57.07±6.14
ALT (iu/l)	58.72±3.43^{ab}	45.68±5.23^{a}	62.12±1.89^{b}	51.22±3.73^{ab}	50.65±5.42^{ab}
AST/ALT	0.98±0.06	1.30±0.20	1.04±0.12	1.20±0.06	1.13±0.05
ALP (iu/l)	215.33±17.88^{c}	189.18±44.13^{bc}	156.73±31.40^{abc}	92.91±18.46^{a}	122.63±16.90^{ab}

Different superscripts ^{abc} along the row denote significant difference at P<0.05

Notes: Values are expressed as means ± SEM in the table

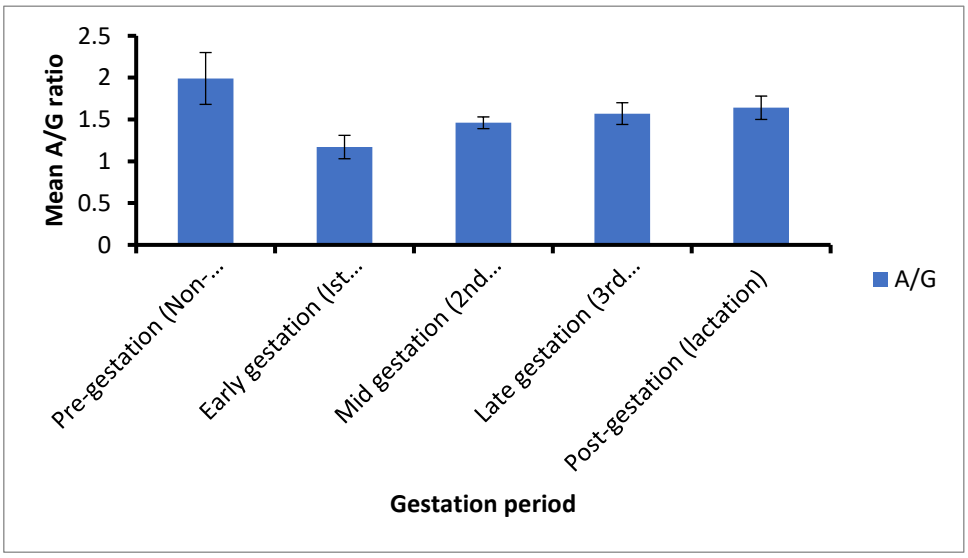

Figure I: The mean serum albumin/globulin (A/G) ratio of sows at different stages of reproduction

Values are expressed as means ± SEM. Values with different superscripts are statistically (p<0.05) different

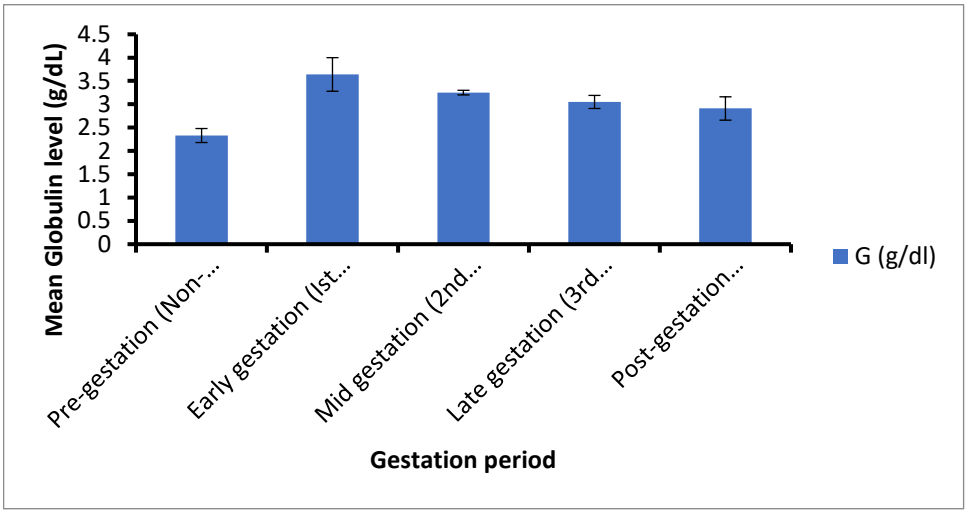

Figure II: The mean serum globulin concentrations of sows at different stages of reproduction

Values are expressed as means ± SEM. Values with different superscripts are statistically ($p<0.05$) different

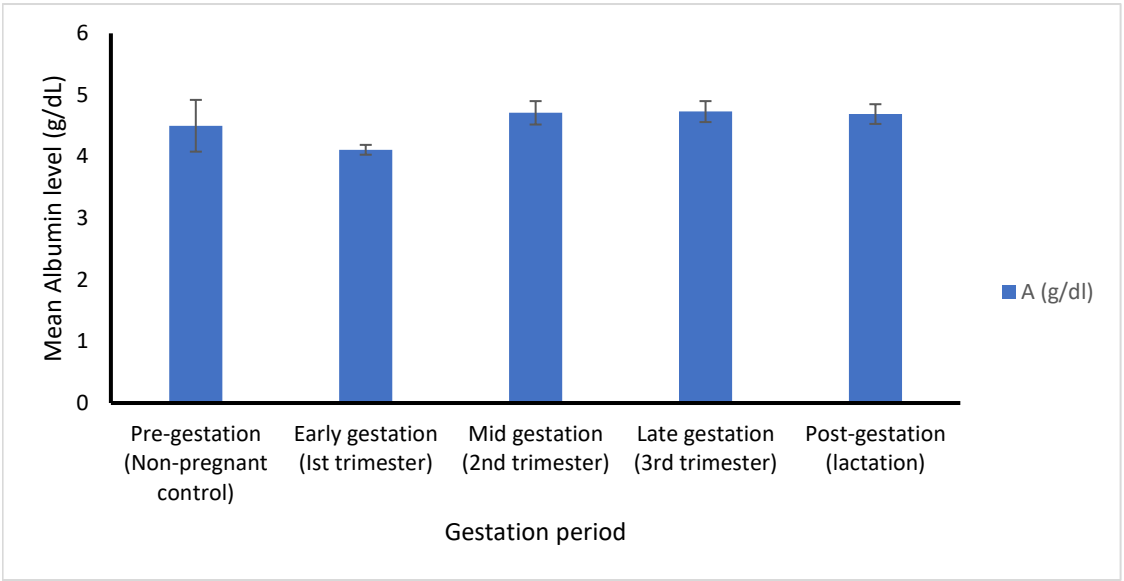

Figure III: The mean serum albumin levels of sows at different stages of reproduction

Values are expressed as means ± SEM. Values with different superscripts are statistically ($p<0.05$) different

were obtained in pregnant and post-kidding goats
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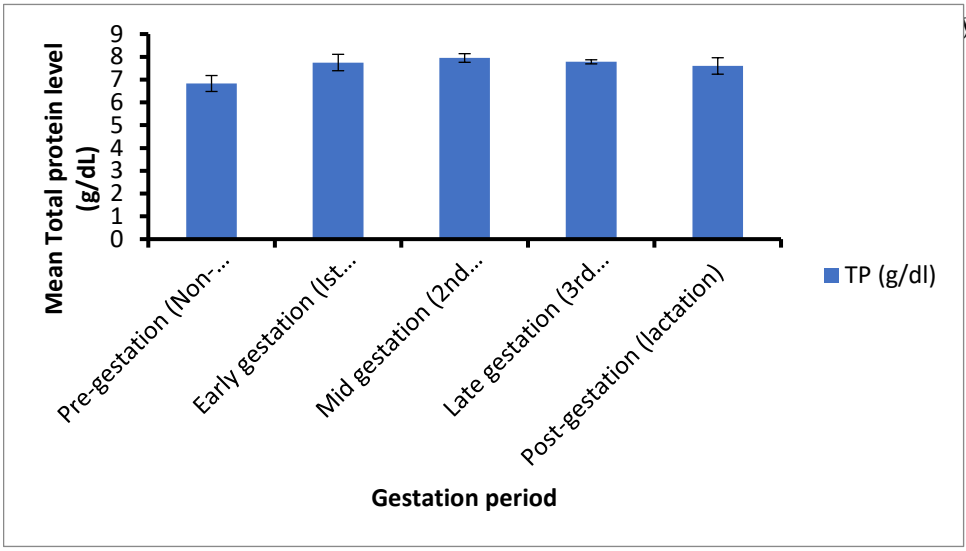

Figure IV: The mean serum total protein levels of sows at different stages of reproduction Values are expressed as means ± SEM. Values with different superscripts are statistically ($p<0.05$) different

DISCUSSION

The biochemical parameters are important for many body functions and their deficiency impaired these functions and causes nutritional disorders (Mcdowell, 1992). Pregnancy and lactation in sows have led to altered metabolism in animals (Krajnicakova *et al*., 2003) and during lactation the mammary gland secretory cells utilize about 80% of blood components for milk synthesis (Bonev *et al.*, 2012). The results of serum liver enzymes of LW sows in this study are shown in table 1 while the results of immunity (protein) parameters of LW sows in this study are presented in figures 1-4. The lowest values of albumin found in early pregnancy in this study could be related to the decreased production of this protein as a result of the elevated productions of some globulin fractions. The mean albumin concentration of pregnant sows in this study was insignificantly ($p > 0.05$) lower compared to postpartum group. Similar results breeds (Zubcic, 2001; Celi *et al.*, 2008; Castagnino *et al.*, 2015). However, this is in contrast to the works of EL-Tarabany (2012) and Manat *et al.* (2016) who reported decreased serum albumin concentrations during late gestation in an Egyptian breed of ewes but agrees with the work of AL-Hassan (2018) who reported also higher albumin concentrations at late gestation. These differences may be related to breed and nutritional differences. Albumin is known to function as a powerful antioxidant (Roche *et al.*, 2008), and this might explain the increased serum albumin concentrations towards the end of gestation seen in this study. Although it is reported that pregnancy toxemia lowers serum albumin concentrations (Vasava *et al.*, 2016), the animals in this study continued to be healthy without any signs of disease. When these results are put together, it appears that blood Albumin was directed to foetal growth tissue, while globulin may be used mainly

for milk synthesis, as occurs in ewes (Balıkcı *et al.,* 2007; Karapehlivan *et al.,* 2007; Obidike *et al.,* 2009). The mean serum globulin concentrations in this study varies significantly ($p < 0.05$) across the groups and this disagrees with the work of Sahu *et al.* (2019) who reported no significant variation in the globulin levels during gestation in Ganjam goats. Globulin can be also used for the formation of milk protein (casein) as suggested by Sharma *et al.* (2015). The high globulin concentration in this study during pregnancy disagrees with the decreasing trend recorded for globulin (Antunovic *et al.*, 2011; Bamerny, 2013).

Metabolism of protein plays an important role in the regulation of physiological functions during pregnancy and lactation (Diogenes *et al.*, 2010). But as previously observed (Djuricic *et al.*, 2011), we observed a reduction in serum total protein levels during lactation and dry periods compared to mid pregnancy. This could be due to the transfer of immune globulins (γ-globulins) and amino acids from the maternal bloodstream to the mammary gland used for colostrum and milk synthesis. This protein fraction (γ-globulins), that provide passive immunity to neonates, accumulates in the bloodstream before moving into the mammary glands where it is fundamental in the colostrum synthesis. The immunoglobulins (Igs) represent the major protein fraction of colostrum accounting for 70–80% of its total protein content and are the result of selective accumulation from serum immunoglobulins (Igs) that starts before parturition (Korhonen *et al.,* 2000). The mean values of TP decreased significantly ($P<0.05$) with advancing gestation period where the lowest concentrations were observed in late gestation period. Balikci *et al.* (2007) and Gürgöze *et al.* (2009) reported a decrease in TP on day 120 to 150 of gestation in ewes compared to other stages of gestation. In accordance, El-Sherif and Assad (2001) and Taghipour *et al.* (2010) reported that TP gradually decreased during pre-partum period and reached the lowest level at parturition and then slowly increased afterward. These reports disagree with the findings of this study. Similarly, Amer (2016) reported reductions in the total protein during pregnancy and this disagrees with our findings.

Data available for the impact of gestation on the ALT and AST activity are unclear and are somewhat controversial (Gürgöze *et al.,* 2009). In a few studies, a decrease in AST and ALT activities have been reported by Manish-Mahawar *et al.* (2004). Khatun *et al.* (2011) reported that serum ALT levels decreased significantly with advancing pregnancy and this is in agreement with our findings. Similarly, serum ALT activity showed a tendency to decrease in late gestation in the Angora cat in the study of Simsek *et al.* (2015) where the serum ALT activity had decreased on day 55 of gestation, in comparison to the value measured on day 15 of gestation and this report agrees with our findings. El-Sherif and Assad (2001) reported that during pregnancy in pregnant Barki ewes, AST started to increase significantly reaching maximum values at parturition and this report disagrees with our findings.

The significant decrease in ALP activity during late gestation compared to the non-pregnant control in this study disagrees with the work of Tran (2005) who reported an increase in ALP activity during late gestation. The significant reduction in ALT activity during early gestation in this study disagrees with Zvorc *et al.* (2006) who reported a significantly reduced ALT activity during lactation compared to non-pregnant control. The authors concluded that increased metabolic rate after parturition, together with possible energy

loss, may lead to cellular hypoxia. This, as a consequence, has increased cell membrane permeability and leaking of the enzymes out of the cell. There was an increased AST/ALT activity in the early gestation sows compared to other groups. An increased AST/ALT ratio is associated with progressive impairment of liver function (Giannini *et al.,* 1999).

CONCLUSSION

The serum liver function markers and protein parameters of LW sows in this study were significantly influenced by the reproductive stages. These factors should be considered when interpreting these parameters during diagnosis and prognosis in order to ensure accuracy in these species. Blood parameters are good indicators of pregnancy in which the body adjusts its physiological and homeostatic mechanisms to ensure the foetus(es) grow properly and receive adequate nutrition. Conclusively, pregnancy caused marked variations in the serum liver function and protein parameters of sows during gestation in this study. Therefore, elevations or reductions in these parameters can be used for complementing the diagnosis of pregnancy after non- return to estrus in large white (LW) sows.

The serum liver function and protein parameters of sows used in this study are within the physiological range required for the maintenance of homeostasis and health essential for efficient reproduction. Therefore, the sows used in this study during gestation suggest having good blood compositions capable of supporting optimal reproductive performance. This study has established reference values of the serum liver function and protein parameters of LW sows during pregnancy and lactation that are consistent with normal health in these species.

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AUTHOR'S CONTRIBUTION

This work was an aspect of Ph.D thesis of the first author and supervised by the second and third authors while the fourth author was the internal examiner of the first author. Authors Nweze Ernest Chukwuamachago, Ikechukwu Reginald Obidike and Samuel C Udem designed the experiment and performed the laboratory procedures. All authors took part in writing and final approval of the manuscript.

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