

## The effects of fetal growth on maternal body weight and visceral organ mass in the grasscutter, *Thryonomys swinderianus*, Temminck

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### SUMMARY

During pregnancy, maternal metabolism changes to support the gravid uterus and the timing of these changes are a function of both gestational length and fetal number. In order to predict the nutrient requirements throughout pregnancy, it is necessary to know the changes which occur in both the reproductive and maternal tissues. Fifty-seven (57) wild grasscutters were used to investigate the increased nutrient requirement of fetal growth on reproductive and maternal tissues in the grasscutter. The animals were categorized into non-pregnant, early pregnancy (d 1 – 50), mid pregnancy (d 51 – 100), and late pregnancy (> d 100) status. Maternal organ weights were reported as fresh weight (g), scaled to empty body weight (EBW; g/g), and maternal body weight (MBW; g/g). Carcass weight declined with advancing pregnancy ( $P = 0.029$ ), as were the lungs, small and large intestines whilst the weights of the kidneys, liver and remaining viscera increased. The total internal organ mass, due mainly to the significant increases in the 'remaining viscera' mass, which is made up of mainly adipose tissues, also increased with advancing pregnancy. From mid pregnancy to late pregnancy, uterine weight increased by 675.3% ( $52.33 \pm 6.525$  v.  $353.37 \pm 26.580$ ). This suggests that pregnancy imposes a huge metabolic demand on the grasscutter, but the animals appear to mobilize maternal nutrient stores, as well as slow down metabolic rate (as indicated by the increases in the fat component of the remaining viscera mass) to meet requirements of the growing fetus and developing mammary glands.

Keywords: fetal growth, grasscutter, maternal body weight, metabolic demand, pregnancy, visceral organ mass

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## INTRODUCTION

Pregnancy results in a number of physiological adaptations in the maternal body, and though maternal system responses to the demands of pregnancy are not well understood, several authors (McNeill *et al.*, 1997, Scheaffer *et al.*, 2004 and Ji *et al.*, 2005) have reported that the biological priority of reproduction (i.e. gestation, fetal growth, and mammary development) may compete for nutrients more successfully than maternal tissues thereby impacting the gastrointestinal tract mass. Battaglia (1992) reported that during a 5-d maternal fast, amino acid uptake across the umbilicus was unchanged illustrating the priority of nutrient delivery to the gravid uterus.

During pregnancy, maternal metabolism changes to support the gravid uterus, and the timing of these changes are a function of both gestational length and fetal number (Noblet *et al.*, 1985, Freetly and Ferrell, 1998, Freetly and Leymaster, 2004). Thus, in order to predict the nutrient requirements throughout pregnancy, it is necessary to know the changes, which occur in both the reproductive and maternal tissues (Noblet *et al.*, 1985), such as knowledge of the dynamic nature of fetal metabolism (Freetly and Leymaster, 2004). This requires the accurate definition of the pattern of fetal and total conceptus growth during pregnancy (Bell *et al.*, 1995).

Scheaffer *et al.* (2004) have stated that fetal growth is a complex dynamic process that depends on the continuous supply of nutrients from the mother, with the ability of the maternal system to deliver a normal fetus being dependent on the capacity of the maternal system to meet uterine nutrient demands. Adam *et al.* (1988a,b), Bell *et al.* (1995), McNeill *et al.* (1997) and Ji *et al.* (2005) have all shown that consideration of actual nutrient accretion in the gravid uterus and nonuterine maternal tissues can allow for the estimation of nutrient needs of animals during pregnancy, with changes in weights and composition in maternal and fetal tissues during gestation being essential for this estimation. This information is however lacking in the grasscutter. Proportions of visceral organs have also been reported to increase with advancing pregnancy in ewes fed restricted or maintenance diets (Scheaffer *et al.*, 2004), which is indicative of the preservation of a functional tissue mass to support the development of the conceptus. There is however, no information on how the GI tract and other visceral tissues respond to the nutrient demands of gestation in the grasscutter. This study was therefore aimed at investigating the proportional changes of visceral organs with advancing pregnancy in the grasscutter.

## MATERIAL AND METHODS

### *Animals*

Fifty-seven (57) wild grasscutters at different reproductive status, collected from hunters' kills, were used to evaluate maternal body changes, i.e. mobilization of maternal nutrient stores, in response to the increased nutrient requirement of fetal growth. Each animal was assigned a serial number and the bled-out carcass weight noted to the nearest  $\pm 10$  g using a digital electronic balance prior to being dressed.

### *Tissue Collection*

Animals were eviscerated and the entire organ tagged similarly to the animal from which it was obtained, stored over ice and transported to the CSIR-Animal Research Institute laboratory for further analysis. Each viscus was dissected into the various organs. Intestinal tissues were located, and the demarcations of small and large intestines made. After specific regions were identified, the mesenteric membranes and visceral fats were dissected away from the tissue; digesta gently stripped, and the segment washed thoroughly with water, blotted dry using paper towels and weighed. The liver was dissected out of the visceral tissues and weighed. The lungs, heart, kidneys, and spleen were also weighed after being dissected out of surrounding visceral tissues and blotted dry. The heart was cut open and blood washed out before drying. All other remaining organs (including pancreas and bladder) and visceral fat were combined as 'remaining viscera'. The weight of the carcass, including hide and head, was defined as the empty bodyweight.

The reproductive tract was dissected from the vagina at the cervix and weighed. The number of implanted embryos, resorption sites and the number of surviving fetuses counted. Fetuses were obtained from the uterine horn by cutting the placenta at the base of the amniotic sac and then at the base of the umbilicus, and individually weighed. Individual fetal and total fetal weights were noted.

### *Calculations and Statistics*

Data from Jin *et al.* (1994) and Swanson *et al.* (2000) have both suggested little difference in fresh and dry weights of tissues. Tissue weights were therefore reported as fresh weights. Empty body weight (EBW) was the summed weight of the carcass (including head, hide and feet), lungs, spleen, heart, kidneys, stomach, intestinal tissue, mesentery, visceral fats and gravid uterus. Empty BW is often used to evaluate the contribution of organ mass to metabolism of animals of different body sizes (Koong *et al.*, 1985, NRC, 1996, Sainz and Bently, 1997).

Empty bodyweight is intended to be a measure of metabolically active tissue and is ideally defined as bodyweight less gut fill. However, when considering nutrient delivery by pregnant animals, the uterine compartment should be considered metabolically active tissue (Scheaffer *et al.*, 2004), and was therefore included in EBW. Maternal bodyweight (MBW) was calculated as EBW with the exclusion of gravid uterine weight (Scheaffer *et al.*, 2004). Expression of data on EBW and MBW bases allows comparisons with other data sets (Burrin *et al.*, 1990). Small intestinal weight included the duodenum, jejunum, and ileum, and large intestinal weight included the colon, cecum and rectum. The digestive tract was the summation of the intestines and the stomach. Total internal organ weight was the summation of the lungs, spleen, heart, liver, kidneys, stomach, intestinal tissue, mesentery, visceral fats and gravid uterus.

The stage of gestation was estimated from the weight of the embryo or fetus using the formula derived by Huggett and Widdas (1951):

$$W^{1/3} = a(t - t_0)$$

where  $W$  = mean weight of fetus,  $a$  = the specific fetal growth velocity, an estimate of which was obtained from Adu and Yeboah (2000) as 0.042,  $t$  = the stage of gestation (in days).

An estimate of  $t_0$  was obtained from Huggett and Widdas (1951) as:

$$t_0 = 0.2 \times \text{gestation length (days)}$$

Data were then categorized into non-pregnant (NP), Early- (d 1 – 50), Mid- (d 51 – 100) and Late-gestation (> d 100) and analyzed as an ANOVA using the GLM procedure of SPSS (SPSS 15 *software* package for Windows; SPSS, Inc, Chicago, IL). Planned comparisons of non-pregnant (NP) vs. Early- (d 1 – 50), Mid- (d 51 – 100) and Late-gestation (> d 100) were used to evaluate the effect of advancing gestation.

## RESULTS

Body weight did not differ between reproductive status. Carcass weight however declined with advancing pregnancy (Table 1). Empty body weight increased with advancing pregnancy ( $P < 0.0001$ ), with maternal body weight following a similar trend (Table 1).

Gravid uterine weight, expressed as a ratio of body weight or empty body weight increased with advancing pregnancy, but was largely unchanged

throughout pregnancy when expressed as a ratio of maternal body weight. Crown rump length was also affected by reproductive status, increasing with fetal growth (Table 2). Fetal number was not different between mid and late pregnancy (Table 2).

Table 1: The effect of reproductive status on body weight change in mature grasscutters

Item	Reproductive status				P
	Non pregnant	Early pregnancy	Mid pregnancy	Late pregnancy	
n	10	19	9	19	
BW, kg	2.91±0.240 <sup>a</sup>	2.94±0.174 <sup>a</sup>	2.79±0.253 <sup>a</sup>	3.16±0.164 <sup>a</sup>	0.232
Carcass wt, kg	2.48±0.049 <sup>a</sup>	2.35±0.036 <sup>b</sup>	2.34±0.052 <sup>a,b</sup>	2.06±0.036 <sup>c</sup>	0.029
EBW, g	164.38±26.794 <sup>a</sup>	188.44±19.438 <sup>a</sup>	239.63±28.243 <sup>a</sup>	560.16±19.438 <sup>b</sup>	0.0001
MBW, g	158.01±16.796 <sup>a</sup>	172.59±12.185 <sup>a</sup>	187.29±17.705 <sup>a,b</sup>	206.78±12.185 <sup>b</sup>	0.052

BW = Bled-out body weight; Carcass wt = eviscerated body weight; EBW (empty body weight) = the sum of total internal organs and carcass weight; MBW (maternal body weight) = EBW – uterine weight; Means within a row with different superscript are significantly different.

Table 2: The effect of reproductive status on uterine weight, fetal weight, and fetal crown-rump length in mature grasscutters

Item	Reproductive status				P
	Non pregnant	Early pregnancy	Mid pregnancy	Late pregnancy	
n	10	19	9	19	
Gravid uterine weight					
- g	6.37±1.049 <sup>a</sup>	15.85±2.163 <sup>b</sup>	52.33±6.525 <sup>c</sup>	353.37±26.580 <sup>d</sup>	0.0001
- g/g BW	2.37 ±0.368 <sup>a</sup>	5.45±0.749 <sup>b</sup>	19.34±2.964 <sup>c</sup>	113.17±8.246 <sup>d</sup>	0.0001
- g/g EBW	0.039±0.005 <sup>a</sup>	0.085±0.010 <sup>b</sup>	0.216±0.020 <sup>c</sup>	0.623±0.025 <sup>d</sup>	0.0001
- g/g MBW	0.041±0.005 <sup>a</sup>	0.095±0.013 <sup>b</sup>	0.286±0.041 <sup>c</sup>	1.864±0.177 <sup>d</sup>	0.0001
Fetal no.	–	4.0±0.27 <sup>a</sup>	3.3±0.15 <sup>b</sup>	3.4±0.10 <sup>b</sup>	0.04
Fetal weight					
- Individual, g	–	0.13±0.045 <sup>a</sup>	6.75±1.168 <sup>b</sup>	89.575±3.572 <sup>c</sup>	0.01
- Total, g	–	0.54±0.465 <sup>a</sup>	19.45±4.808 <sup>b</sup>	284.30±23.664 <sup>c</sup>	0.0001
CRL, cm	–	1.13±0.488 <sup>a</sup>	4.60±0.276 <sup>b</sup>	11.17±0.178 <sup>c</sup>	0.0001
BMI, g/cm <sup>2</sup>	–	0.072±0.033 <sup>a</sup>	0.256±0.019 <sup>b</sup>	0.702±0.012 <sup>c</sup>	0.0001
PI, g/cm <sup>3</sup>	–	0.071±0.006 <sup>a</sup>	0.057±0.003 <sup>b</sup>	0.064±0.002 <sup>a</sup>	0.042

EBW (empty body weight) = the sum of total internal organs and carcass weight; MBW (maternal body weight) = EBW – uterine weight; CRL (crown rump length) = the length of the fetus from the crown of the head to the tailhead; BMI = body mass index, which equals BW/CRL<sup>2</sup>; PI = ponderal index, which equals BW/CRL<sup>3</sup>; Means within a row with different superscript are significantly different.

Changes in heart and lung weights with reproductive status are presented in Table 3, whilst changes in spleen, liver, and kidney weights with reproductive status are presented in Table 3. Heart weight and lung weight were unaffected, on a whole organ basis, by advancing pregnancy (Table 3). Spleen weight, liver weight and kidney weight, on the other hand, increased in response to advancing pregnancy. However the initiation of pregnancy resulted in a decrease in spleen weight; thereafter the increasing response of

spleen weight to the growing conceptus led to spleen weights for non-pregnant and animals in late pregnancy not being significantly different (Table 3). With the exception of spleen weight, which increased, when expressed as a ratio of maternal body weight, all other organs decreased with advancing pregnancy when expressed either as a ratio of empty body weight or maternal body weight (Table 3).

Table 3: The effect of reproductive status on the weights of the heart and lungs in mature grasscutters

Item	Reproductive status				P
	Non pregnant	Early pregnancy	Mid pregnancy	Late pregnancy	
n	10	19	9	19	
Heart					
- g	9.12±0.813 <sup>a</sup>	10.45±0.590 <sup>a</sup>	9.81±0.861 <sup>a</sup>	9.64±0.595 <sup>a</sup>	0.190
- g/g EBW	0.055±0.003 <sup>a</sup>	0.057±0.002 <sup>a</sup>	0.041±0.003 <sup>b</sup>	0.018±0.003 <sup>c</sup>	0.002
- g/g MBW	0.057±0.004 <sup>a</sup>	0.063±0.003 <sup>a</sup>	0.051±0.004 <sup>a,b,c</sup>	0.049±0.003 <sup>a,b,c</sup>	0.018
Lungs					
- g	15.14±1.448 <sup>a</sup>	17.21±1.050 <sup>a</sup>	17.25±1.533 <sup>a</sup>	16.76±1.060 <sup>a</sup>	0.252
- g/g EBW	0.094±0.004 <sup>a</sup>	0.092±0.003 <sup>a</sup>	0.070±0.004 <sup>b</sup>	0.031±0.003 <sup>c</sup>	0.0001
- g/g MBW	0.098±0.005 <sup>a</sup>	0.101±0.004 <sup>a</sup>	0.900±0.006 <sup>a,b</sup>	0.084±0.005 <sup>b</sup>	0.040
Spleen					
- g	5.46±0.645 <sup>a,c</sup>	4.74±0.468 <sup>c</sup>	6.40±0.680 <sup>a,b</sup>	6.83±0.468 <sup>a,b</sup>	0.003
- g/g EBW	0.034±0.002 <sup>a</sup>	0.026±0.002 <sup>b</sup>	0.026±0.003 <sup>b</sup>	0.013±0.002 <sup>c</sup>	0.036
- g/g MBW	0.035±0.003 <sup>a</sup>	0.028±0.002 <sup>a</sup>	0.033±0.003 <sup>a</sup>	0.035±0.002 <sup>a,b</sup>	0.042
Liver					
- g	31.91±3.231 <sup>a</sup>	39.37±2.343 <sup>a,b</sup>	35.87±3.421 <sup>a,b</sup>	40.91±2.364 <sup>b</sup>	0.029
- g/g EBW	0.192±0.010 <sup>a</sup>	0.213±0.007 <sup>a</sup>	0.147±0.011 <sup>b</sup>	0.077±0.007 <sup>c</sup>	0.003
- g/g MBW	0.200±0.011 <sup>a</sup>	0.232±0.008 <sup>b</sup>	0.188±0.012 <sup>a,c</sup>	0.204±0.008 <sup>a,c</sup>	0.027
Kidneys					
- g	6.94±0.496 <sup>a</sup>	7.97±0.359 <sup>a,b</sup>	8.40±0.525 <sup>b</sup>	8.30±0.363 <sup>b</sup>	0.047
- g/g EBW	0.044±0.003 <sup>a</sup>	0.045±0.002 <sup>a</sup>	0.035±0.003 <sup>b</sup>	0.016±0.002 <sup>c</sup>	0.031
- g/g MBW	0.046±0.003 <sup>a</sup>	0.049±0.002 <sup>a</sup>	0.044±0.003 <sup>a</sup>	0.043±0.002 <sup>a</sup>	0.075

EBW (empty body weight) = the sum of total internal organs and carcass weight; MBW (maternal body weight) = EBW – uterine weight. Means within a row with different superscript are significantly different.

Stomach weight, and the weights of the small and large intestines, on a whole organ basis, did not respond to the growth of the conceptus; but decreased when scaled to either empty body weight or maternal body weight (Table 4). Table 4 shows trends in the weights of the remaining visceral mass, digestive organ mass and total internal organ mass. Remaining viscera mass increased with advancing pregnancy. Total internal organ weight increased on a whole organ basis ( $P \leq 0.035$ ), when expressed as a ratio of maternal body weight ( $P = 0.043$ ), but decreased when scaled to empty body weight, in response to advancing pregnancy.

Table 4: The effect of reproductive status on digestive tract organs in mature grasscutters

Item	Reproductive status				P
	Non pregnant	Early pregnancy	Mid pregnancy	Late pregnancy	
n	10	19	9	19	
<b>Stomach</b>					
- g	6.48±0.642 <sup>a</sup>	6.02±0.466 <sup>a</sup>	6.16±0.677 <sup>a</sup>	6.63±0.479 <sup>a</sup>	0.370
- g/g EBW	0.039±0.003 <sup>a</sup>	0.033±0.002 <sup>a</sup>	0.026±0.003 <sup>b</sup>	0.011±0.002 <sup>c</sup>	0.048
- g/g MBW	0.041±0.003 <sup>a</sup>	0.036±0.003 <sup>a,b</sup>	0.034±0.004 <sup>a,b</sup>	0.031±0.003 <sup>b</sup>	0.024
<b>Small intestines</b>					
- g	13.68±1.896 <sup>a</sup>	10.69±1.376 <sup>a</sup>	11.18±1.999 <sup>a</sup>	13.50±1.376 <sup>a</sup>	0.154
- g/g EBW	0.081±0.005 <sup>a</sup>	0.056±0.004 <sup>b</sup>	0.047±0.006 <sup>b</sup>	0.025±0.004 <sup>c</sup>	0.002
- g/g MBW	0.084±0.007 <sup>a</sup>	0.061±0.005 <sup>b</sup>	0.060±0.007 <sup>b</sup>	0.066±0.005 <sup>b</sup>	0.034
<b>Large intestines</b>					
- g	32.40±3.105 <sup>a</sup>	27.86±2.252 <sup>a</sup>	32.54±3.273 <sup>a</sup>	28.52±2.252 <sup>a</sup>	0.242
- g/g EBW	0.194±0.011 <sup>a</sup>	0.154±0.008 <sup>b</sup>	0.137±0.012 <sup>b</sup>	0.054±0.008 <sup>c</sup>	0.005
- g/g MBW	0.202±0.014 <sup>a</sup>	0.169±0.010 <sup>a,b</sup>	0.175±0.015 <sup>a,b</sup>	0.144±0.010 <sup>b</sup>	0.001
<b>Remaining viscera</b>					
- g	36.29±9.061 <sup>a</sup>	49.55±6.750 <sup>a</sup>	61.89±9.594 <sup>a,b</sup>	70.41±6.630 <sup>b</sup>	0.032
- g/g EBW	0.211±0.025 <sup>a</sup>	0.227±0.018 <sup>a</sup>	0.244±0.026 <sup>a</sup>	0.130±0.018 <sup>b</sup>	0.012
- g/g MBW	0.219±0.030 <sup>a</sup>	0.246±0.022 <sup>a,b</sup>	0.310±0.031 <sup>b,c</sup>	0.341±0.022 <sup>c</sup>	0.040
<b>Digestive tract</b>					
- g	48.57±5.208 <sup>a</sup>	47.04±3.778 <sup>a</sup>	52.53±5.490 <sup>a</sup>	46.68±3.778 <sup>a</sup>	0.384
- g/g EBW	0.227±0.020 <sup>a</sup>	0.142±0.014 <sup>b</sup>	0.098±0.021 <sup>b</sup>	0.290±0.014 <sup>c</sup>	0.013
- g/g MBW	0.250±0.019 <sup>a</sup>	0.261±0.014 <sup>a</sup>	0.222±0.021 <sup>a</sup>	0.308±0.014 <sup>b</sup>	0.023
<b>Total internal organs</b>					
- g	157.82±15.512 <sup>a</sup>	171.50±11.249 <sup>a,b</sup>	190.47±16.423 <sup>a,b</sup>	199.64±11.352 <sup>b</sup>	0.035
- g/g EBW	0.945±0.024 <sup>a</sup>	0.902±0.017 <sup>a</sup>	0.775±0.025 <sup>b</sup>	0.373±0.017 <sup>c</sup>	0.0001
- g/g MBW	0.983±0.001 <sup>a</sup>	0.986±0.001 <sup>a,b</sup>	0.987±0.002 <sup>b,c</sup>	0.990±0.001 <sup>c</sup>	0.043

EBW (empty body weight) = the sum of total internal organs and carcass weight; MBW (maternal body weight) = EBW – uterine weight; Remaining viscera = the weight of the pancreas, bladder, visceral fat, mesentery and diaphragm. Means within a row with different superscript are significantly different.

## DISCUSSION

### *Body weight*

Animals used for this study were from hunters' kills. Body weight of the animals was thus the weight at the time the animal was killed. It was therefore not possible to determine the pre-pregnant body weight. However it appears there were wide variations within the different categories for the pre-pregnant body weights. This might have contributed to the mean body weights not increasing with advancing pregnancy (Table 1). Though there were no differences in body weight, carcass weight declined with advancing pregnancy, declining from early pregnancy to late pregnancy. This indicates that the maternal system was mobilizing nutrient stores to meet requirements of the growing fetus and developing mammary glands with the onset of pregnancy. Differences in body weight and carcass weight can indicate differences in

energy utilization between groups of animals that differ in physiological states or plane of nutrition (Sainz and Bently, 1997, Scheaffer *et al.*, 2001, 2004). Results from the current study indicate that responses in carcass weights to physiological status, an indication of a transition in maternal metabolism, occur in early pregnancy in the grasscutter (Table 1). This is in contrast to the situation in the cow (Scheaffer *et al.*, 2001). Stock and Metcalfe (1994) also outlined evidence for a transition from anabolic to catabolic phases of gestation as pregnancy advanced.

Naismith and Morgan (1976) demonstrated a transition in carcass protein gain in rats with advancing pregnancy, demonstrating that from conception to d 14 there was a net gain in maternal carcass protein, and thereafter there was a net protein loss. Similar evidence by Lawrence *et al.* (1984) has demonstrated that when metabolizable energy was limited, maternal metabolism decreased in order to meet the nutrient demand of gestation, whereas when metabolizable energy was adequate maternal metabolism increased linearly as gestation advanced.

#### *Uterine variables*

Uterine weight in the current study increased over 5,547% from the non-pregnant status to the late pregnancy status ( $6.37 \pm 1.049$  v.  $353.37 \pm 26.580$ ), and 2,229.5% ( $15.85 \pm 2.163$  v.  $353.37 \pm 26.580$ ) from the early pregnancy status compared to the late pregnancy status. From mid pregnancy to late pregnancy uterine weight increased by 675.3% ( $52.33 \pm 6.525$  v.  $353.37 \pm 26.580$ ). This compares with an increase of 224.7% in the ewe from mid to late gestation (Scheaffer *et al.*, 2004). The ability of the fetus to grow to term is limited by the ability of the maternal system to deliver essential substrates (Scheaffer *et al.*, 2004). The large increases, i.e. about a 3-fold increase, in the gravid uterine weight in the grasscutter, as observed in the present study (Table 2), compared to the situation in the ewe (Scheaffer *et al.*, 2004) suggest that pregnancy imposes a huge metabolic demand on the grasscutter. It is thus imperative to adequately feed the grasscutter with the onset of pregnancy and throughout the period of gestation.

#### *Lungs, heart, spleen, liver, and kidneys*

When scaled to empty body weight, the lungs decreased with advancing pregnancy ( $P < 0.0001$ ). However when expressed per unit of maternal body weight lung weight remained unchanged until late pregnancy, a situation similar to work done in sheep (Scheaffer *et al.*, 2004). This indicates the need of the maternal tissue to maintain a threshold of functional weight to support the increased metabolic needs of conceptus development (Scheaffer *et al.*, 2004).

Nutrient delivery to and uptake by the gravid uterus seems to depend primarily on the rate of uterine blood flow (Reynolds *et al.*, 1985). An examination of changes in heart weight throughout gestation could give an indication of blood flow and nutrient delivery during pregnancy. Stock and Metcalfe (1994) showed changes in maternal blood volume, cardiac output, stroke volume, and heart rate in women, and reported that the left ventricular wall thickness and mass were elevated by the 12<sup>th</sup> week and increased progressively throughout pregnancy.

Heart weight data from the current study (Table 3) showed that heart weight remained unchanged, on a whole organ basis ( $P = 0.19$ ), but decreased with advancing pregnancy when expressed either as a ratio of empty body weight ( $P = 0.002$ ) or maternal body weight ( $P = 0.018$ ). Report by Scheaffer *et al.* (2004) showed that there was a decline in heart weight for ewes on a restricted diet. The grasscutters in this study might therefore have been in a poor nutritional state as they were collected during the dry season (February – March), when the nutritive value of the forages had declined; and this could explain the decline in heart weight observed in the study.

Contrary to reports by Rosenfeld (1977) and Scheaffer *et al.* (2004) who reported a decrease in spleen weight due to pregnancy in sheep, spleen weight for late pregnant animals in the current study was not different from that of non-pregnant animals (Table 3). Heap and Lodge, (1967) and Johnson *et al.* (1985) also had results similar to that observed in the present study; with spleen weight not changing as a result of pregnancy in the sow and rabbit respectively. There could thus be species differences in the response of the spleen to pregnancy.

Liver weight in the current study increased with increasing stage of pregnancy on a whole organ basis, but decreased as a proportion of empty body weight ( $P = 0.003$ ) or maternal body weight ( $P = 0.027$ ) with advancing pregnancy. The liver plays a pivotal role during the adaptation of maternal metabolism to pregnancy, and this is reflected in the changes in weight and function during different physiological state. Pregnancy results in the enlargement of the liver with the nutritional status of the mother influencing this enlargement. During pregnancy, liver sensitivity to dietary nutrient intake may be altered owing to nutrient demand of the gravid uterus. When ewes were fed a restricted diet liver weight was maintained late in pregnancy (Robinson *et al.*, 1978), whilst an approximately 50% of *ad libitum* restriction of food intake in rats resulted in a hampering of pregnancy-induced liver enlargement (Kuriyama *et al.*, 2000).

Pregnancy-induced liver enlargement has been reported to be due to a transient swelling of parenchymal-cells (Borlakoglu *et al.*, 1993), which may be as a result of increases in progesterone concentration. In this regard, Ochs and

Schulte-Hermann (1986) have demonstrated that continuous exposure of non-pregnant rats to high doses of progesterone in the diet causes liver hypertrophy.

In the present study, kidney weight was increased as a result of advancing pregnancy (Table 3). Physiological alterations which accompany normal pregnancy include marked increases in glomerular filtration rate and renal plasma flow (renal haemodynamics) and increments in kidney size (Lindheimer and Katz, 1977, cited by Davison and Lindheimer, 1980). Data by Davison and Lindheimer (1980) showed an increment in renal haemodynamics and renal wet weight in gravid Sprague-Dawley rats from d 8 – 10 of pregnancy though there were no changes in renal dry weight and [<sup>14</sup>C]choline incorporation into cortical phospholipids, a sensitive marker of renal growth, compared to the situation in non-pregnant littermates. Davison and Lindheimer (1980) therefore suggested that pregnancy-induced renal enlargement in the rat was functional rather than structural in nature due to increments in water content and not to accelerated growth. In this study, neither renal dry weight nor [<sup>14</sup>C]choline incorporation into cortical phospholipids was measured. However we speculate that a similar situation might account for the current observation in the grasscutter. Further experiments are necessary to investigate this.

#### *Gastrointestinal tract*

The data in this study show that the small and large intestines combined represent just about 1.4% of body weight, and 1.7% of carcass weight (i.e. empty body weight). These values fall well below the 2.5% and 4.0% quoted for the cow (Scheaffer *et al.*, 2001). The gastrointestinal tract and the liver are responsible for a disproportionately high fraction of whole-body energy utilization (McBride and Kelly, 1990,). The gastrointestinal tract constitutes 4 to 12% of body weight yet consumes 20 to 25% of whole-body oxygen (Kelly *et al.*, 1993). Sainz and Bently (1997) and Nozière *et al.* (1999) have also reported that visceral tissues have respond to dietary nutrient density by direct proportional changes in mass during nutrient restriction. The gastrointestinal tract is thus an important organ to examine when studying factors that affect whole-body metabolism (Scheaffer *et al.*, 2001).

When dietary intake is restricted to the level of limiting increases in body weight, the small intestine decreases in weight, which results in an overall decrease in energy use by the animal. According to Reeds *et al.* (1999) the fractional rate of protein turnover in intestinal tissues exceeds that of peripheral tissues in adults by as much as 30-fold. Scheaffer *et al.* (2001) however reported that responses of intestinal tissues to pregnancy in beef heifers were inconsistent and appeared to be dependent on stage of gestation and segment of the gastrointestinal tract examined. For instance Scheaffer *et*

*al.* (2004) reported increases in the weights of the small intestine, ileum, duodenum, jejunum, digestive tract and total internal organs, with advancing pregnancy, whilst the weights of the intestinal mesentery, cecum, colon and large intestines increased by d 90 of pregnancy compared to the non-pregnant state. Thereafter the weights of these organs decreased to d 130 of pregnancy.

Decreasing levels of dietary intake results in decreases in metabolic rate of the digestive tract (Huntington *et al.*, 1988, Krehbiel *et al.*, 1998) in proportion to body weight (Ferrell and Koong, 1986). In contrast to these reports Robinson *et al.* (1978) have shown a numerical increase in intestinal mass due to advancing gestation in ewes fed a restricted level of intake. However, in most of these cases, the overall body mass requiring nutrients is also less, responding to decreasing levels of intake. In this study, stomach weight decreased when scaled to either empty body weight or maternal body weight in the pregnant compared to the non-pregnant animal ( $P < 0.02$ ; Table 4). A similar situation has been reported in sheep (Scheaffer *et al.*, 2004). Stomach weight has been indicated to be responsive to type of diet (Fluharty *et al.*, 1999) and level of diet (Fluharty and McClure, 1997). The nutritional status of the grasscutters in the current study might have thus mediated the decreasing condition in stomach weight.

The weight of the metabolic organs (i.e. total internal organ mass) increased with advancing pregnancy. In the present study there were decreases in the lungs, small and large intestines whilst the weights of the kidneys, liver and remaining viscera increased. The total internal organ mass also increased with advancing pregnancy. This is due mainly to the significant increases in the 'remaining viscera' mass, which is made up of mainly adipose tissues. Compared to reports by Scheaffer *et al.* (2001, 2004) in which there were decreases in the spleen, kidneys, lungs and small intestines; and increases in liver weight, the increases in the 'remaining viscera' in the current study demonstrates that the grasscutter employs a different adjustment strategy, particularly during the dry season, in order to potentially conserve metabolic fuel or to increase the level of functional tissue to meet the metabolic priorities in support of the growing conceptus. This situation is similar to reports by Lawrence *et al.* (1984) who demonstrated that pregnant women deficient in metabolic energy still deposited fat as a result of a slowing metabolic rate as pregnancy advanced.

#### CONCLUSIONS

This study suggests that pregnancy imposes a huge metabolic demand on the grasscutter, but the animals appear to mobilize maternal nutrient stores, as well as slow down metabolic rate (as indicated by the increases in the fat

component of the remaining viscera mass) to meet requirements of the growing fetus and developing mammary glands. It is thus imperative to adequately feed the grasscutter with the onset of pregnancy and throughout the period of gestation.

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