CHAPTER ONE

INTRODUCTION

1.1.0 General Introduction

While there is pressure to find socially acceptable ways of reducing the reproductive capacity of the exploding human population, there is great need to maintain reproductive performance in our domestic animals at maximum capacities to provide adequate nutrition (and other useful animal products) for the rapidly expanding human population (Hansel and McEntee, 1970). Ghana's population grows at a rate of 2.7%[1] and so may require a corresponding adequate supply of meat to meet the demand. In Ghana, goats provide about 30%[2] of ruminant meat supply from cattle, sheep, and game with goat meat (or chevon) being relatively more preferred in the urban centers (Odoi and Amevor, 2003). Although the expected demand for goats is great, the average annual growth of goats in Ghana is only 5%, with estimated total population of 3,923,482 by the close of 2005[2]. The need then to raise the goat population level through reproduction cannot be overemphasized.

1.2.0 Nutrition, Flushing and Reproduction

Raising and maintaining the reproductive performance of domestic animals to their maximum capacities require a good knowledge/understanding of all phases of reproduction to enable practical manipulation at the farm level. One such practical attempt has been nutritional flushing – a nutrition interaction believed to affect the body condition and fertility of the dam just before or during mating.

Nutritional flushing has been reported since the start of the twentieth century, but usually from temperate countries for sheep (Robinson, 1973), cattle (McCann and Hansel, 1986), and only recently for goats (Hart *et al.*, 1999), though without very firm or absolute conclusion on its economic effectiveness (Miller and West, 1972).

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[1] Ghana Population Census (2000). Ghana Statistical Services, Accra.

[2] National Livestock Census (1996). Livestock Population Unit, Ministry of Food and Agriculture, (MoFA), Accra.

The few studies on nutritional flushing reported in Ghana, focused on ewes, but produced inconclusive results (Brown, 1971; Larsen *et al.*, 1975; Blasu, 1981)

Research to understand the mode of the link between nutrition and reproduction has exploded over the last decade of the twentieth century especially in domestic animals, as reviewed by Webb *et al.* (2004). It may be suggestive from the works of Meza-Herrera *et al.* (2004) in goats; McCann and Hansel (1986) in Cattle and Bellows *et al.* (1973) in sheep that the effects of nutrition on reproduction in farm animals, may be associated with the metabolism of insulin, leptin, growth factors, glucose (energy), and protein; which in turn affect pituitary-ovarian function. Thus, Bellows *et al.* (1973) postulated that since pituitary gonadotropins are proteinaceous in nature an attempt to elucidate the physiologic basis of flushing in ewes might be via determination of protein deposition and assay of subsequent hormonal levels. It follows then that the link between flushing and prolificacy in female animals may be traced with determination of the metabolic and pituitary-ovarian functioning status at mating. The metabolic status may be characterized by blood levels of protein, metabolic fuels such as glucose, and metabolic hormones such as insulin; while that of the pituitary-ovarian activity by blood levels of the gonadotropins (luteinizing hormone, LH and follicle stimulating hormone, FSH) and female sex hormones (estradiol and progesterone).

The current study seeks to link prolificacy with rising plane of nutrition, based on the hypothesis that a change in overall metabolism that favors increased protein synthesis may eventually lead to higher pituitary potency and, subsequently, increased ovarian stimulation and activity of the doe at mating, resulting in multiple ovulation with possible multiple-birth.

1.3.0 The Study Objectives

This study has the ultimate objective to determine a link between nutritional flushing and prolificacy in does (female goats) through increased pituitary-ovarian activity. It seeks specifically to:-

1. Monitor in West African Dwarf (WAD) does the effects of nutritional flushing on their:

(A). Physical and metabolic status at mating characterized by body condition score and serum levels of glucose, total protein, calcium and insulin
(B). Pituitary functioning status at mating with respect to serum levels of FSH and LH
(C). Prolificacy, measured as litter size per conception

2. Determine statistical interrelationships (if any) among the observed indices of the physical/ metabolic and pituitary-functioning status at mating and the prolificacy.

3. Make appropriate recommendations.

CHAPTER TWO

LITERATURE REVIEW

2.1.0 Introduction

In reviewing data on reproductive performance of the doe, information from sheep is assumed where data on goats are not available or need further evidential support. This is because in many respects (except length of estrus and prolificacy) the characteristics of the reproductive cycle are similar between the two species (Wildeus, 1996). In addition, related data from other livestock and mammals in general are alluded to where necessary.

The profitability of chevon production enterprise is closely related to total kid output of the flock in their productive life (Ezekwe and Lovin, 1996, cited by Sodiq *et al.,* 2003). This in turn is largely a function of two main components: the number of offspring born (known as prolificacy) and the frequency with which they are produced i.e. kidding frequency (interval) (Alderman and Cottrill, 1995; Wildeus, 1996). The most profitable does are therefore those that have long productive persistency; that is, does that attain puberty early with optimum body condition or live weight at first service (Devendra and McLeroy, 1992), are prolific (Hafez, 1993), have short kidding intervals and long biological life (Fall *et al.,* 1982).

For the purpose of enterprise-budgeting, Pinkerton *et al.* (2000) consider the length of productive flock life for well-managed temperate does to be five years, or a 20% per year breeding flock disappearance rate; and 5 to 7 years for bucks if healthy and reproductively sound. Kinne (2000) mentioned a reproductive life of 4-8 years for Pigmy does in USA. Of utmost importance to a goat farm enterprise is rearing a flock that can generate enough replacement for them, expand flock size and supply excess stock for sale (Peacock, 1996) through reproductive improvement. Good reproductive performance is therefore a prerequisite for any successful livestock production programme (Abassa, 1995). The components of reproductive performance – prolificacy and parturition frequency – are influenced by both genetic and environmental factors.

When considering ways to improve reproductive performance, it is important first to recognize the genetic limitations (Walker *et al.,* 1994). Heritability of reproductive efficiency is very low, between 0 and 25 percent (Devendra and McLeroy, 1992; Peacock, 1996) in goats. This means that additive gene effect is minimal and that genes that affect fertility may be those with non-additive effect. In cows, genetic selection of reproductive traits is limited due to strong effect of environment on the traits (Walker *et al.,* 1994). Poor reproductive efficiency then may only be temporary within a flock, being the result of periodic adverse environmental conditions (Bearden and Fuquay, 1984; Walker *et al.,* 1994). The environmental factors include climate, nutrition, parasites, pathogens and management (IAEA, 1989; Devendra and McLeroy, 1992). However, careful husbandry practice such as good sheltering, supplementation and regular veterinary attention may modify the effects of some aspects of climate, nutrition and health, respectively (Devendra and McLeroy, 1992). Hence, the environment could be essentially reduced to the product of the management system and the uncontrollable climatic factors (Wilkins, 1986). So with its low heritability and high responsiveness to environmental influence reproductive efficiency of the doe, as in cow, may be improved practically to a large extent through sound management techniques especially the overall nutrition of the animal (IAEA, 1989; Walker *et al.,* 1994), which must be consistent in the productive year round and not limited to pre-breeding flushing period (Hart, 2005 – personal communication). Naturally, any such managerial biotechnique may have to be directed at either or both of the components of reproductive performance – parturition frequency and prolificacy.

Manipulation of reproduction by genetic, physiologic, and environmental methods could increase the frequency of breeding per year and the litter size in species with seasonal reproduction (Hafez, 1993). However, in most tropical breeds, and hence WAD goats, reproduction is largely aseasonal (Devendra and McLeroy, 1992; Hafez, 1993). This means does can be bred all through the year, and with a frequency rate of twice a year[3] or thrice-in-two-years as observed in studies (Awotwi and Fynn, 1992;

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[3] My own doeling, during the period of this study, kidded twice in 12 months: singleton in the first kidding followed by twins, affirming possibility of twice-a-year frequency. It was well fed all through with a steady body condition of about 2.8

Hafez, 1993; Abassa 1995; Odoi and Amevor, 2003) or reported at Agricultural Stations (MOFA-APD, 2004). According to Ashton *et al*. (1970) cited by Awotwi and Fynn (1992) and ILCA (1987) the 3-in-2 years frequency should be preferred if the doe must not be physiologically overstressed. Thus considering the physiologic welfare of goats, WAD does seem to have already attained a limit of kidding frequency, and it appears that this limit is the highest for small ruminants in Sub-Saharan Africa (SSA), though 5 kidding in 3 years is also reported (Mba, 1979, cited by Akusu and Ajala, 2000). In that case any managerial method, including nutritional techniques, to improve reproductive efficiency of WAD does may have to be directed more at the prolificacy than the kidding frequency.

2.2.0 Prolificacy of the Female Animal

2.2.1 Definition of Prolificacy

Prolificacy is one of the measures of reproductive efficiency in small ruminants. It is defined as the number of progeny born per parturition (Haumersser and Gerbaldi, 1980, cited by Abassa, 1995). According to Abassa (1995) other definitions that specify that the progeny need to be alive at birth (Hafez, 1993) may not be appropriate. That live-birth must not be specified in the definition of prolificacy may be of physiological importance because it deemphasizes an anticipation of only economic gains of reproduction at the expense of the does' physiological wellbeing as implied in such definitions. The fact is that does that deliver stillborn kids also carry pregnancy and suffer almost the same physiological distresses like live-kid deliverers, and must be so recognized as to be considered in any appropriate postpartum physiological fitness plans for does kidding. Moreover, other factors such as poor pregnancy and or parturition management rather than problems with the doe's reproductive system *per se* may be the cause of kid losses. Abassa (1995) stated that fetal loss can be caused by two groups of factors; and these are of environmental (diseases, nutrition, season, dam age, litter size, and management) and genetic origins. Therefore, non-live parturitions may not have to be disqualified when computing prolificacy as an index of the dam's reproductive efficiency. Perhaps it is for this reason or among reasons why some earlier definitions imply measuring prolificacy as per conception (pregnancy) (Gunn and Doney, 1971) rather than per parturition.

Generally, prolificacy is a term used to describe the capacity of the doe to produce many kids (Devendra and McLeroy, 1992) following one mating season or in a given breeding interval, such as one year (Hafez, 1993).

2.2.2 Indices of Prolificacy

2.2.2-1 Litter Size and Percent Kidding

Litter size is a measure of the number of progeny a female animal delivers from each pregnancy or parturition. It can be used for either the individual dam and indicated, for instance, as kids per doe; or for the flock, in which case it is calculated as the average number of kids per conception or parturition within a breeding interval. According to Abassa (1995) average litter size for a flock can be calculated on a yearly basis to be consistent with the annual rate of fertility. However, it is said that for tropical breeds displaying aseasonal estrus or, when goats are kept under controlled conditions, litter size may be computed per kidding (Devendra and McLeroy, 1992). Abassa (1995) reported a comprehensive review of estimates of goat prolificacy values across Sub-Saharan Africa (SSA). Overall, SSA's native goats produce litter sizes per doe between 1.05 and 1.87 kids. The highest range (1.84 to 1.87) is for WAD does from Agricultural Stations in the humid zone of Ghana (Boadu, 1972; Otchere and Nimo, 1976). Recently Karbo *et al*. (2003) and Baffour-Awuah *et al*. (2005) reported litter sizes of 1.5 and 1.3 for WAD does under extensive traditional systems at Bawku (semi-arid zone) and semi-intensive system at Kintampo Goat Breeding Station (transitional humid /semi-arid zone), respectively in Ghana. These values are lower than both the earlier ones from the same country collated in Abassa's review (1995) and an average range of 1.6 - 1.8 stipulated by Steel (1996) for tropical breeds. In a 4-year study at Ibadan, a humid tropical zone in Nigeria, Akusu and Ajala (2000) obtained litter size of 1.91 kids / doe for WAD does. Peacock (1996) indicates that for even the acclaimed highly prolific WAD doe litter sizes per conception normally range between 1.6 - 1.8 kids when computed as number of kids per doe. Akusu and Ajala (2000) reported litter size of 1.9 for WAD does in the humid environment of Ibadan in Nigeria. None of these litter sizes is close to the estimated maximum potentials of 4 to 6 kids per doe.

For purposes of international uniformity and comparative analyses, prolificacy is measured also in a unit of annual percentage or kidding rate: that is the number of kids per 100 kiddings (Abassa, 1995) or per 100 does (Devendra and McLeroy, 1992) in a flock in a year. According to Peacock (1996) it is important to define the does used in the calculation as either those actually mated or just the potential breeders in the flock. When expressed as percentage, Abassa (1995) observed that SSA's native does exhibit prolificacy values ranging from 149 to 187; 118 to 171; 105 to 162 and 135 to 175 percent in the humid, sub-humid, semi-arid zones and highlands, respectively.

2.2.2-2 Multiple birth rates

Implicit in the definition of prolificacy is the expectation of multiple births. The rate of multiple births is calculated as percentage of total parturitions during a breeding (parturition) interval. For native goats in SSA, Abassa (1995) collated only twinning rates, which commonly varied from 13.5 percent (for Sahel goats in semi-arid zone in Niger) to 60.4 percent (for WAD goats in humid zone of La Côte d'Ivoire). On zonal basis, the humid zone values varied from 51.12 percent for WAD goats in Ghana (Oppong and Yeboah, 1981) to 60.4 percent for WAD goats in La Côte d'Ivoire. Akusu and Ajala (2000) obtained twinning rate of 60.3% for WAD in humid zone of Nigeria. In the sub-humid zone, twinning rate is between 16.4 and 59.0 percent; and the highest in the whole SSA being the 59.0% for WAD breed in Ghana (Oppong and Yeboah, 1981). Reports from the semi-arid zone showed twinning rates of 47.5 percent and 56.7 percent for Red Sokoto/Maradi (Nigeria) and Landim (Mozambique) goats, respectively (Abassa, 1995). In the recent findings of Karbo *et al.* (2003) and Baffour-Awuah *et al.* (2005), twinning rates of 43.7 and 50.2 percent were reported for WAD does at Bawku (semi-arid zone) and Kintampo (transitional zone), respectively, in Ghana. The report for Kintampo also showed a triplet rate of 1.6% (Baffour-Awuah, 2005). Generally then, it may be said with some degree of confidence from the preceding observations that WAD does are relatively a high prolific tropical breed. According to Abassa (1995) the twinning data for the sub-humid and humid zones of SSA confirm the well accepted findings (FAO, 1980; Hofs *et al.*, 1985; Gatenby, 1986) that WAD breed is highly prolific and stands up well among the prolific tropical breeds. Their records of 59 and 60.4 percent twinning rates imply that they shed at least two ova per parturition in about every 60 out of 100. This compares well with many well known prolific breeds such as the Kambing Katjang of Malaysia (66 percent) and the Barbari in India (69 percent) both quoted from Devendra and Burns (1970) by Abassa (1995).

2.3.0 Components of Prolificacy

According to Gunn and Doney (1971), prolificacy is determined by two complementary components: *Ovulation rate* - the number of ova shed in the estrous cycle; and *prenatal wastage* or the total loss of potential progeny from ovulation to parturition (Edey, 1969, cited by Abassa, 1995).

2.3.1 Ovulation Rate

Ovulation rate – whether natural/normal or artificially induced - sets the upper limit for prolificacy (Hafez, 1993; Walkden-Brown, 2001). The normal ovulation rate is the number of ova shed in any one estrous cycle within the peak of a normal breeding season by a doe in natural physiological state uninfluenced by treatments with exogenous hormones (Gunn and Doney, 1971). The difficulty in its measurement may be because procedures for direct determination of the number of ova shed by a female animal are often costly or highly technical. They involve slaughter of the animal (Maracek *et al.,* 2002), surgical laparotomy and laparoscopy (Hafez, 1993), transrectal ultrasonography scanning (Meza-Herrera *et al.,* 2004) and in vivo water-flushing of reproductive tract after breeding (Hafez, 1993). It is costly and time consuming to measure pre-embryo loss in Sub-Saharan Africa (SSA) (Abassa, 1995); and this then may be the reason affecting the determination and or reporting of normal ovulation rates also.

The use of such highly technical and costly devices and methods to determine ovulation rate in developing countries may be economically prohibitive unless in heavily funded research projects. Thus, at best ovulation rate in normal farm situations may only be indirectly estimated by inference from the litter size at birth, though such estimates may be potentially inaccurate. Hafez (1993) finds the possible inaccuracy in cases where a doe with multiple-pregnancy loses some of the embryos early in the process but carries the remainder to term without the lost one(s) ever being detected. Generally, sheep and goats are said to be monotocous (giving birth to one progeny at a time) (Frandson, 1974). However, in many of their breeds two or more ova are shed during estrus (Hafez, 1993; Walkden-Brown, 2001). In a study, about 50.2% multiple births (twins and triplets) were observed by Baffour-Awuah *et al.* (2004) suggesting that WAD does, considered as one of the most prolific tropical breeds (Abassa, 1995) are capable of releasing, at least, two or three ova in an estrous cycle. It has been estimated that prolificacy of 4 kids (Wildeus, 1996) per pregnancy is the biological ceiling of litter size – and hence reflective of natural ovulation rate – in goats. Kinne (2000), reports that some Pygmy goats have even produced six healthy kids and, hence, six or more ova being shed at a time. In practice, however, real litter sizes are often lower than the estimated biological ceilings in most breeds. Litter size at birth can help establish the minimum number of successful (fertilizable) ova shed in a particular mating period. However, multiple ovulations *per se* are no guarantee of high prolificacy if there is high rate of prenatal wastage.

2.3.2 Prenatal Wastage

According to Abassa (1995) despite the several definitions provided by earlier workers, confusion over the identity of prenatal wastage seems to remain a problem. Adopting that of Edey (1969), he defined prenatal wastage (mortality) as total losses occurring from ovulation to parturition including death of the ovum be it fertilized or not.

In an attempt to identify the components of prenatal wastage Wilmut *et al.*, (1985) concluded that fertilization involves the successful fusion (syngamy) of the sperm and the ovum. The embryo is the conceptus from the moment of syngamy to the time of implantation and the fetus is the conceptus from implantation to parturition. Pregnancy has three periods which are the period of ovum, that of the embryo and that of the fetus. The period of ovum, also known as pre-attachment or pre-implantation period, is the time from fertilization to the first loose attachment of the blastocyst to the endometrium and is thought to last until day 14-15 in sheep (Edey, 1969; Kelly, 1984). The embryonic period lasts until about day 40 when the differentiation of the embryonic parts and placenta (organogenesis) is concluded (Edey, 1976; Abassa, 1995). Hafez (1993) stated that the ovum period ends on day 12 while embryo period is from day 12-34 in sheep.

Accordingly, death during the period of ovum can be reasonably referred to as early embryonic loss whilst that occurring during the embryonic period can be named late embryonic loss. A ewe will have a cycle of normal length if the embryos die before day 12 when maternal recognition of pregnancy occurs (Moor and Rowson, 1964). Edey (1967) pointed out that embryo mortality occurring before this recognition day is unlikely to be distinguished from failure of fertilization. Abassa (1995) defined embryo mortality as death of fertilized ova (or embryos) before the completion of organogenesis and fetal mortality to deaths occurring from the end of organogenesis to birth; and therefore, prenatal wastage should encompass fertilization failure, embryo mortality and fetal death. Losses at these stages then become the components of prenatal wastage and are reflective of his definition except that the contribution of ovulation failure implied in his definition is not taken care of in the component of "fertilization failure" which is sequential to ovulation failure. It follows from the preceding argument that the designation "fertilization failure" is physiologically limiting in describing early reproductive losses occurring from the end of ova production in the ovary, 'handing over' of ova to the ovarian end of the tubular genitalia and formation of the zygote (single cell embryo)[Hafez,1993].

It seems that other events such as ovulation and egg pick-up which precede fertilization also incur losses that are significant to the reproductive efficiency of the female animal in terms of prolificacy – although they occur within a relatively shorter period (in minutes, Hafez, 1993) and less frequently in practice than others that follow. Consequently, in this review while adopting the definition and components of prenatal wastage as given by Abassa (1995) the component "*fertilization failure*" is re-designated "*pre-embryo losses*" to be more physiologically inclusive of losses during the swap over from egg to commencement of progeny production. Prenatal wastage then is due to pre-embryo losses, embryo mortality and fetal deaths.

2.3.2-1 Pre-embryo Losses

This may be defined as that part of female reproductive failure involving losses due to unsuccessful physiological events occurring from ovulation to fertilization. It consists of failures or disorders of ovulation, ovum pick-up and fertilization.

2.3.2-1.1 Ovulation failures

Fertilization takes place in the oviduct. The ovum must therefore be successfully ovulated (i.e. expelled) from the ovary into the oviduct. Failure of ovulation in any one estrous cycle renders the reproductive process wasteful by abruptly ending that breeding cycle from estrus to lactation (Frandson, 1974). In dairy cattle and aged sows absence of ovulation and the subsequent formation of follicular cysts (cystic ovary) are the main causes of reproductive failure (Hafez, 1993).

Ovulation failure may occur in two forms: *anovulation* and *cystic ovaries*. Anovulation is a failure of the Graafian follicle to rupture during normal cycle. It is noted also that even after rupturing some follicles may still entangle and detain the oocytes inside them. Cystic ovary, on the other hand, is a disease condition of the ovaries, induced by endocrine abnormality, by which one or both ovaries contain multiple cysts (small or large) which fail to ovulate and either regress (in the case of large ‘follicular’ cysts) or persist for a prolonged period (in the case of small ‘luteal’ cysts). According to Hafez (1993) ovulation failure generally is less common in sheep, goats and beef cattle, but may be caused by deficiency in GnRH or LH; or (in the case of cattle) insensitivity of the hypothalamo-pituitary axis to elevated levels of estradiol.

2.3.2-1.2 Failure of ovum pick-up

The ovum, even after ovulation, may never reach the oviduct. This is because of difficulties in picking-up of the egg from the ovarian surface; and this itself may be due to causes such as egg entrapment in the ruptured follicle (developing corpus luteum), loss of egg into the peritoneal cavity (sometimes resulting in ectopy) and malfunctioning of the egg pick-up mechanism of the oviduct (Hafez, 1993). The malfunctioning of the egg pick-up mechanism may result from various factors including the structural characteristics of the fimbriae of the infundibulum and its relationship to the surface of the ovary at the time of ovulation. For example, adhesions of the infundibulum to the ovary or uterine horns may interfere with egg pick-up or cause a mechanical obstruction of the ovarian end of the reproductive duct (Hafez, 1993). It appears that the metabolic status of the doe at estrus may also affect the egg pick-up mechanism. This is because the oviductal contractions (including those of the fimbriae kinocilia, which ‘massage’ the ovarian surface) are believed to be associated with glycogen content of the oviductal musculature (Hafez, 1993).

It follows then, that only an ovum that successfully is located in the oviduct after ovulation stands the chance of normal fertilization. But this chance may be lost through fertilization disorders.

2.3.2-1.3 Fertilization disorders

The disorders of fertilization include ‘failure of fertilization’ and atypical (abnormal) fertilization. Fertilization failure may result from death of the egg before sperm entry, structural and functional abnormality in the egg or sperm, physical barriers in the female genital tract preventing gamete transport to the site of fertilization or ovulatory failure (Hafez, 1993). In sheep some of the conception failures at the beginning of the breeding season are associated with a high incidence of abnormal ova such as giant egg, oval-shaped egg, and egg with ruptured zona pellucida. In cattle where the physiologic significance of abnormal sperm in relation to fertilization failure has been studied more than other species (Hafez, 1993) abnormality in sperm include defects of the DNA protein complex, sperm aging and injury to the acrosomal cap (apical end of the sperm head). It appears that as a natural provision to avoid defective spermatozoa fertilizing the egg acrosin, an enzyme within the acrosome, which helps the sperm to penetrate the zona pellucida of the egg, becomes inhibited in only sperms with damaged acrosome (Hafez, 1993).

In the case of atypical (or aberrated) fertilization, the commonest example is polyspermy (entry of more than one sperm). This may happen as a result of egg aging or delayed copulation, which lowers the biophysical and biochemical reactions that regulate sperm entry. According to Hafez (1993) embryos resulting from polyspermic fertilization are triploid (have three times the chromosomal number of the species) and do not survive. This means that when estrus is prolonged, timing of mating in relation to ovulation is critical for normal fertilization.

There is inadequate information on the effect of pre-embryo losses on the prolificacy of small ruminants, especially in developing countries. According to Abassa (1995), there is no published report on fertilization failure in sheep and goats of SSA, perhaps because this source of losses is costly and time consuming to measure. Nonetheless, caprine reproduction is known to be a very wasteful process in view of the large difference between potential production and progeny produced. Kelly (1986), cited by Abassa (1995) stated that while a ewe can only produce five to ten offspring in her lifetime, she could produce about 4,000 lambs if all the potential eggs in her ovaries developed. Restall *et al.* (1976), quoted in Willingham *et al.* (1986) found that 30 percent of infertility in adult ewes and 34 percent in maiden ewes were ascribed to a failure of the ewe to mate successfully. A fertilization failure rate of 30 percent has been assumed for sheep and goats in Africa (Charray *et al.,* 1980 cited in Abassa, 1995).

2.3.2-2 Embryo Mortality

Embryo mortality denotes the death of fertilized ova and embryos up to the end of implantation (Hafez, 1993).It comprises basal or normal (i.e. unknown-factor-caused) and factor-influenced losses.

The failure of a fertilized egg to develop normally has been investigated by many workers. Edey (1969) and Wilkins *et al.* (1984) cited in Abassa (1995) concluded that 20-30 percent of embryos are normally lost during pregnancy with most losses occurring in the first 30 days. The normally occurring embryonic death is called basal embryonic mortality. The loss occurs under normal and stress-free conditions. Hafez (1993) states that in sheep and cattle normal embryo loss is 25-40% between the time of sperm penetration of the ovum and the end of implantation; and that most embryos die between days 9 and 15 after mated ovulation. Abassa (1995) affirms that the questions as to why such loss occurs even in well-managed flocks and why no factor or combination of factors investigated and controlled have not been able to eliminate normal embryo loss, remains unanswered. Bishop (1964) cited by Abassa (1995), stated that the basal/normal embryonic death might be a perfectly normal way of eliminating unfit genotypes at low biological cost (i.e. without wasting much needed energy for full term pregnancy and parturition).

Hanley (1961) suggested that there is some universally active factor which seems to be responsible for the relatively constant residual death of fertilized ova. Edey (1969) believed that until such a factor is discovered, many embryos will continue to die within the first 30 days of pregnancy in their native environment: the uterus. However, Wilmut and Sales (1981) think it could be that asynchrony between ewe and embryo causes the latter to become abnormal, and be expelled from the uterus, which is unable to prevent luteolysis (Lawson *et al.*, 1983, cited by Abasssa, 1995). Maternal factors, embryonic factors or embryonic-maternal interactions can also cause embryonic mortality (Hafez, 1993). Maternal failure tends to affect an entire litter and hence complete loss of pregnancy; but embryonic failure affects individual embryos only. Another possible reason advanced by Hafez (1993) is insufficient maternal environment, allowing the support of only a few strong embryos. All these findings suggest that it is still not possible to put a finger on any clearly identified biophysiological situation or reason why apparently normal fertilized ova develop for some time and then naturally die in their native environment. Recently, however, Lucy (2003) concluded from an experiment with embryo transfers, involving normal and repeat-breeder cows that, seemingly normal embryos may fail to develop within the uterus because the uterus may not synthesize adequate amounts of embryotropic growth factors that are required by filamentous embryos.

It is now also generally accepted that identifiable factors exist which cause embryo death rate to rise beyond the basal limit; and that these factors include genotype, dam age, nutrition, hormonal conditions, and site of ovulation, ovulation rate, litter size, temperature and quality of semen, diseases, and immunologic incompatibilities (Abassa, 1995). As a case for the dam's nutritional level, Mapletoft *et al.* (1986) cited by Lucy (2003), examined pregnancy rates after embryo transfers and found that recipients with low body condition score (BCS) had lower conception compared to recipients with high BCS and confirms that the nutritional status of the dam at mating and early gestation may contribute to embryo mortality/survival.

Periods of particular vulnerability for the embryo include early cleavage (Dust, 1963), the time of maternal recognition i.e. day 12 (Edey, 1969), and day 18 when the transfer from yolk sac to allantoic placentation occurs (Robinson, 1951; Moor *et al.*, 1960; Quinlivan *et al.*, 1966 (all cited by Abassa, 1995). Generally, more than half of the losses occur before day 13 and most of the remainder by day 18 according to Edey, (1967; 1969; 1976). Robinson (1951) notes that loss after commencement of implantation may simply be a continuation of a process initiated long before. Nalbandov (1976) seems to agree when he states that any zygote, embryo or fetus destined to suffer prenatal mortality may have this fate determined even before fertilization; and that this is because ova lose their ability to survive as embryos before they lose the ability to become fertilized and cleave.

Thus, it may be concluded that even if the biophysiological factor that preconditions embryos for normal mortality is yet unknown, there are known genetic, climatic and management factors that aggravate the predisposition at vulnerable stages to increase the embryo mortality rate. Apparently, the probability of loss of embryo after implantation is relatively reduced unless it has already been destined by the ‘unknown factor’ to perish.

Reference values for small ruminant embryo mortality rates in SSA are few, however, Abassa (1995) mentioned a report in which abattoir materials were used, and which claimed early embryo deaths of 3.6 percent in Malian goats (Wilson and Traore, 1988). He, however, comments that abattoir material has many shortfalls such as the impossibility to distinguish between failure of fertilization and early embryo death, and the resulting under estimation of the latter. Edey (1969) had expressed similar opinion when he stated that where early embryonic mortality and resorption have occurred, a corpus luteum might still be seen and such cases cannot be differentiated from cyclic corpora lutea in unmated ewes.

2.3.2-3 Fetal Deaths

Fetal deaths refer to gestational losses from the end of implantation (and organogenesis) at day 30-40, through to parturition. Abassa (1995) conludes, in a review, that under normal conditions loss of conceptus during this fetal period is small; it is less than or equal to 6 percent. Hafez (1993) stated that in sheep, most losses between implantation and weaning occur during the perinatal period (around parturition time) and that enzootic abortion in ewes occurs during the last month of pregnancy, and continues until normal parturition time. The main contributions to fetal loss are abortion and stillbirth (Abassa, 1995), which are greatly worsened by disorders of pregnancy and parturition (Hafez, 1993).

The difference between abortion and stillbirth has been explained. From Abassa’s review (1995) abortion is the expulsion before full term of a conceptus unable to sustain independent life (Dennis, 1969) or death *en utero* prior to the start of normal birth process (Woolliams *et al.,* 1983). Being more specific with time Hafez (1993), describes abortion as termination of pregnancy with the expulsion of a fetus of recognizable size before it is viable, which is arbitrarily defined as 260 days for cattle, 290 days for horses and 110 days for swine. Stillbirth, on the other hand, is the expulsion of a dead conceptus that is fully developed and may normally be expected to survive outside the uterus if born live (Wooliams *et el.,* 1983).

The foregoing views suggest that fetuses that suffer abortion or stillbirth are often well developed morphologically before death. But those destined to abortion apparently lack acquisition of physiological viability before a critical time assigned in days arbitrarily as 260 for cattle, 290 for horses and 110 for swine (Hafez, 1993). Peacock (1996) stated that in does, spontaneous abortions occur mostly between days 90 and 115 of gestation. My personal observations at Kintampo Goat Breeding Station in Ghana during this study showed that the few fetal abortions among the experimental WAD does occurred about 6 days before kidding (i.e. around day 139). Agbenyega[4] (personal communication, 2005) expects that, in human beings, fetuses that lack viability before week 28 may be more susceptible to spontaneous abortion, with the most critical time being between weeks 8 and 12. Hafez (1993) states that at least 90% of human conceptuses that are chromosomally abnormal at fertilization will be lost spontaneously before 20 weeks of gestation.

Thus, it may be suggested that, perhaps, as in the case of *embryo* mortality, a small percentage of *fetal* loss is normal, unavoidable and therefore spontaneous in reproductive process of farm animals, usually through abortion. Such normal abortions during fetal period in farm animals may be associated with a time-limited development of physiological viability status, and not to prepartum death. According to Hafez (1993), fetal death is not an essential prelude to abortion. Therefore, a fetus may escape its destiny of abortion but will die perinatally because it loses ability to survive as a neonate before it loses survival prepartum. Hafez (1993) indicates that with Enzootic Abortion of Ewes (EAE) some fetuses may be expelled alive at term but are so diseased and die shortly. Essentially such fetuses must be regarded physiologically already dead before being born alive. Undoubtedly, such kids may be

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mistakenly counted as normal or live-deliveries; or at best included in early neonatal deaths after dying eventually.

It follows from these preceding views that there seems to be a critical time limit, *en utero*, for attaining fetal viability status *ex utero*, which is towards late gestation (early to mid trimester), with specific variations in species, breed and individuals, in both farm animals and humans. Hafez (1993) stated that most fetal abortions in sheep occur getting close to parturition time. Peacock (1996) believes that spontaneous abortion is probably slightly more common in goats than in other female domestic animals; and that most of these abortions normally occur between days 90 and 115 of gestation.

Monty and Racowsky (1987) cited by Hafez (1993), observe that, an environmental factor such as heat stress is able to cause spontaneous abortion of one-week old embryos in dairy cows because it decreases the viability and development capacity of the embryos. Therefore, any factor that interferes with fetal development and the capacity to attain extra-uterine survival before the critical time may cause spontaneous abortion. According to Abassa (1995), the intrinsic propensity of does to abort spontaneously is worsened by extrinsic factors such as nutrition, season, doe age, litter size, management, diseases and disorders of gestation.

In his review of fetal loss estimates in SSA, Abassa (1995) had difficulty in comparing values on abortion and stillbirths between breeds, species, and management conditions within ecological zones. This is because of paucity of data coupled with varying classification or expression of the values. He refers to authors like Ambruster (1988), who expressed fetal loss based on percent of total breeding females as the right ones. Other measurements include:

a) Percent of total progeny born (Wilson and Traore, 1988)
b) Percent of parturitions (Murayi *et al.*, 1985)
c) Percent of live births (Adesiyun *et al.*, 1983)
d) Percent of all fetuses (Suleiman, 1976)
e) Percent of pregnancies (Akakpo, 1988)

Nevertheless he finds estimates expressed as percent of breeding females which range from 13 to 40 for goats to appear very high compared with those ranging from 3.7 to 13.65 reported for sheep by Okoh (1986), Ambruster (1988) and Bourzat (1980). In the humid and sub-humid zones abortions have been estimated at 25 percent of breeding does and 3.7 to 6.5 percent of breeding ewes (Armbruster, 1988, cited in Abassa, 1995). In summary most abortion rates are higher than the estimate of 9 percent of breeding females for the western and central African sheep and goats (Charray *et al.,* (1980).

Abassa (1995) observed that available estimates on stillbirths *per se* are fewer than those on abortions in SSA and offer no basis for comparison. However, there are values of 10.3, 14.9 and 10.64 percent reported by Wilson and Traore (1988), Suleiman (1976) and Osuagwuh *et al.,* (1980). These are high when approximated to estimates of 3.8 and 4 percent obtained for sheep in Cameroon and Mali (Branckaert, 1987; Wilson and Traore, 1988), and 7.39 percent reported in Nigeria (Osuagwuh and Akpokodje, 1984).

Abassa (1995) observed also that losses due to fetal death in general had rarely been investigated and that stillbirths have been neglected or generally recorded as abortions. He, however, concluded from the scanty available data that among ewes and does in SSA, abortion rates are likely to be high and considerably variable (3.7 to 40 percent of breeding females). Goats are more prone to abortions than sheep. Except in few cases the rates are exceedingly high when compared with the tolerable levels of 1-5% (Watson, 1952) and 9% (Charray *et al.,* 1980) estimated in earlier works cited in Abassa (1995).

2.4.0 Factors affecting Prolificacy

The factors that affect the doe's prolificacy are genetic and environmental – the latter in turn comprises climate, dam age and parity, and management (including health, nutrition and use of biotechniques) (Hafez, 1993; Abassa, 1995). These factors interact within and between groups (Abassa, 1995). Therefore, the determination of their individual effect is obviously complex as it is difficult to consider the influence of any one factor alone in a series of interactions at a time (Gunn and Doney, 1971). This complexity is becoming clear with advanced research technologies, and it is now known that apart from interactions between environment and genotype, interaction between environmental factors themselves is also important (Martin *et al.,* 2004). The combinations of these factors are best analyzed holistically; and though some useful and interesting work has occurred in sheep in this area the literature for goats is relatively thin – especially in SSA (Abassa, 1995). On the whole, with the development of modern research technologies such as in improved hormone assays, molecular and cellular biology and use of real-time ultrasound, several of the environmental influences on reproduction are being better understood than before (Martin *et al.*, 2004). Although current available information is useful in developing new management systems to improve animal productivity much more remains to be known about how or by which mechanism these genetic and environmental factors affect reproductive rates (Martin *et al.*, 2004).

2. 4. 1 Effect of Genetics

Animal genotype determines potential productivity such as reproductive and growth rates. Genes control the rates at which reproductive phenomena occur by controlling the rate at which reproductive hormones are secreted. All hormone-controlled reproductive phenomena are therefore subject to genetic selection. Ovulation rate, as a reproductive phenomenon, is partly determined by genetically expressed hormones (gonadotropins, steroids, growth factors and prostaglandins) that regulate the number of follicles developing in the ovary and rupturing at estrus (Peacock, 1996; Spearow *et al.,* 1999). Even though goats, like sheep, are generally classified as monotocous (Nalbandov, 1976) breed differences in ovulation rate occur (Hafez, 1993; Kinne, 2000). West African Dwarf does are known to be potentially multiple pregnancy bearers while Sahel does are known for single pregnancies (Abassa, 1995). According to Draincourt and Cahill (1984) cited by Hafez (1993) it is likely that different control systems operate to generate a high ovulation rate in prolific breeds. For instance, in sheep, it is established that a single gene – the fecundity gene (Fec^b) - influences the high prolificacy of Booroola ewes (Spearow *et al.,* 1999; Kinne, 2000). It does so by extending the time during which follicle recruitment (growth from 2mm to at least 5mm diameter size) takes place, reducing the incidence of dominant follicle(s) and enabling ovulatory follicles (ready to rupture) to wait for the LH peak (required to stimulate ovulation) (Hafez, 1993).

Normally, once a dominant follicle is selected, it suppresses and denies competing subordinate follicles in the cohort opportunity to ovulate. The subordinate follicles then undergo atresia. The Fec^b gene seems to regulate this dominant suppression in a way that *allows* some of the competing second largest follicles to escape atresia and qualify for ovulation by the time LH surge reach its peak (threshold) for ovulatory stimulation. In this way, the probability for multiple ovulations is enhanced by both the selection of *additional* ovulatory-size follicles from the subordinates, and non-atresia of the original dominant follicle despite delay in ovulatory LH stimulation. Apparently, there is a critical time by which large well-developed antral follicles can be destined to respond to LH stimulation for ovulation or else become atretic. From a study on sheep, Matton *et al.* (1977) found that, for multiple ovulation to occur, the second largest follicles must attain the minimum pre-ovulatory or selective size [which according to Maracek *et al.* (2002) is about 5mm diameter] at a time less than 4 days before LH surge. Hafez (1993) stated that, in cows, the ovulatory follicle (usually one) can be identified by its size about 3 days before the onset of estrus, when there are one or two (non-growing) large follicles on the ovaries. It means if these other large follicles are allowed to continue their growth they may attain ovulatory size within the 3 days before LH surge reaches its peak in estrus. This *allowed time* is what Fec^b buys for the second largest follicles to be selected also for ovulation and thereby increase the ovulation rate.

In contrast, many genes control ovulation rate in Ramnov ewes (Kinne, 2000). And unlike the “time buying” approach of the Booroola fecundity gene (Fec^b), the genes that control prolificacy in Ramnov sheep increase ovulation rate by influencing recruitment of higher than usual number of antral follicles between days 13 and 15 of the estrous cycle (Hafez, 1993). In this way, more than the usual few follicles may attain ovulatory size at the time, increasing the chance for multiple ovulations. Thus while genetic effect is established at follicle recruitment stage in Ranmov sheep, it is operative at both recruitment and selective stages in Booroola sheep.

Nalbandov (1976) suspected that the genetic effects on ovulation rate are mediated through differences in the rate of gonadotropin secretion. It is now established that there is a positive association between ovulation rate and follicle stimulating hormone (FSH) in blood plasma around the time of ovulation in very prolific breeds (Hafez, 1993; Kinne, 2000).

Prolificacy, measured either as litter size or rate of multiple births, is also affected by genetics. In SSA Abassa (1995) observed twinning rates for does in the semi-arid ecozone and under village management systems as 13.5 – 44.1, 21.37 and 29.75 percent for Sahelian strains, Swaziland native goat and Zimbabwean Small East African goats, respectively. Similarly twinning rates for the goat breeds reported from the sub-humid zones also differ. Under traditional systems, strains of WAD breed have values ranging between 34 and 45 percent while for the Newala (Tanzania) and Manny (Uganda) breeds the values are 36 and 32.7 percent, respectively. Thus although most studies have shown low values for heritability and repeatability of prolificacy (not exceeding 0.2 to 0.3 in caprines) (Owen, 1971), the influence of genotype can make different breeds under the same environmental condition to display varying ovulation rates, inferable from their prolificacies. In cattle, Kirk *et al*. (1982) cited by Hafez (1993) affirmed that *cystic ovarian disease* (which causes ovulation failure) is inheritable because the incidence steadily declined in several herds after culling bulls whose daughters had cystic ovarian disease.

Similarly, in the case of embryo and fetal mortality animal genotype is among the important factors affecting them (Abassa, 1995). In sheep many authors (Foote *et al*., 1959; Bellows *et al.,* 1963; Cumming *et al.,* 1975; Edey, 1976 and Mayer *et al.,* 1983 – all cited by Abassa, 1995) generally agreed that significant differences in embryo mortality exist between breeds and lines. From data presented by Bourzat (1980) for sheep and goats in the semi-arid zone of Burkina Faso, Abassa (1995) observed that Mossi type animals in this zone aborted more during the hot season than Peul type breeds, which are closely related to the WAD breed (Charray *et al.,* 1980). Osuagwuh *et al.* (1980) have also presented evidence that some genotypes of native breeds may do better than others in a given environment. In a crossbreeding trial involving WAD, Yankasa and Uda sheep, they observed higher stillbirths for Yankasa x WAD (22 percent) and Uda x WAD (23 percent) than for pure WAD breed (0 percent).

It is not known for sure how genotypes induce prenatal wastage though lots of useful information are available. According to Hafez (1993), several abnormalities of male and female reproduction are genetically determined, although the precise genetic mechanism is not well understood. It is, however, generally accepted that genetic disorders may either be inherited or caused by chromosomal aberrations (Hafez, 1993) usually leading to ovulation and fertilization failures, as well as embryo and fetal abortions and stillbirths which are in turn due to resultant conditions such as incompatible blood types, lethal gene effects from inbreeding and inherited predisposition for dystocia (Kinne, 2000). The inherited genotype of the male (sperm) may include a variety of genetic factors that lead to incompatibility and early embryonic loss (Hafez, 1993).

Variations in prenatal wastage among individuals within and between breeds exist (Abassa, 1995); and according to Hanrahan (1983), who found low heritability and repeatability estimates (O.11 ± O.04 and 0.06 ± 0.07 respectively) for the trait in Merino ewes, the potential for direct selection for higher embryonic survival may be small. Abassa (1995) suggests that where breed or genotype differences and genotype x environment interactions are detected, possibilities for improvement exist and must be used to reduce prenatal losses.

2. 4. 2 Effects of Climatic Factors

The important climatic elements to which sheep and goats are particularly sensitive during mating and parturition seasons are light, temperature, rainfall and humidity (Hafez, 1952). The literature on the mechanism and effects of climatic elements on sexual phenomena of small ruminants is either conflicting, or not heavily documented, except for photoperiod, which influences timely ovulation in females with seasonal estrus; and high temperature, which negatively affects embryo survival (Edey, 1969). For tropical goats it is established that their estrus is photoperiodically aseasonal (Mamabolo and Webb, 2005). Therefore, not duration of light, but other elements such as season, temperature, rainfall and humidity are the more relevant climatic factors affecting reproduction (Mamabolo and Webb, 2005). However, not much is reported on how season, rainfall and humidity directly affect prolificacy, as compared to temperature (Table 2.1). The scanty reports on season and rainfall often invariably relate to their indirect effect of causing variations in availability of feed (Amoah and Gelaye, 1990).

In a study to determine the effects of seasonal forage supply on some fertility parameters in the Small East African (SEA) goat in northern Kenya, Rutagwenda *et* *al*. (1985), reported that animals mated prior to the long rains (March-May) in 1984 and kidded at the beginning of the long dry season (June-September), showed the highest conception and kidding rates, with no abortions (Table 2.1). Those mated at the end of the long dry season had the lowest conception and kidding as well as the highest abortion rates. They concluded that high conception rates and low embryo mortality were favored by wet season mating, and negatively affected by the dry season. McKinnon and Rocha (1985) working on Nguni sheep and Landim goats of Mozambique reported that while the better mating period for sheep seemed to be March (rainy season), the worst being the dry season from April to July, goats seemed to be less affected by season of mating, though the best results were attained during the dry season mating. They explained that the difference between the sheep and goats was due to their different feeding habits. Sheep being predominantly grazers should be more affected by the drop in nutritional value of the native grasses during the dry season. Goats' browsing behavior should allow them to maintain a better nutritional status as a result of utilizing leaves and husks from shrubs and small trees, coupled simultaneously with being less affected by gastro-intestinal parasites. While Rutagwenda *et al.* (1985) considered that wet season mating was better McKinnon and Rocha (1985) could not observe any effect of season of mating, but suggested that the dry (rather than wet) season mating might be more favorable. However, the discrepancy appears to be an error either in the recording or interpretation of the results of McKinnon and Rocha (1985) since their narrative does not seem to agree with the tabulated result. For from their table the best mating season for goats was December to March, which, as reported for sheep, was described as rainy or wet season.

It is indicative from both studies that, the effect of season on prolificacy of these native does is mediated mainly through its influence on feed availability at various stages of the doe's reproductive cycle rather than the season *per se.* According to Charles (1985) season is a composite of environmental effects, and in the tropics the most obvious is the relationship of rainfall with pasture, which is difficult to separate in a cause-and-effect-analysis (Valencia *et al.*, 1990).

Apart from season's effect through availability of feed, it may affect female reproductive efficiency directly through stresses of variations in temperature.

Mellado and Meza-Herrera (2002) present an abstract of their study of the influence of season and environment on fertility of goats under intensive conditions in the hot-arid environment of northern Mexico, involving Alpine, Toggenburg, Granadino and Nubian does. They reported that the warmest season with average maximum ambient temperature of between 34^0C and 36^0C was associated with high conception rate (70% in Summer) compared to the cooler seasons (62% and 64% for Spring and Autumn respectively), when average maximum temperature was less than 34^0C 3 days before mating. Conception rate of does bred during the rains was 14 percentage points lower ($P<0.01$) than mating during dry season. It is clear then that for the does in this study warm and dry seasons favored ovulation rate and fertilization more than cool and rainy seasons. They concluded that rain at mating depresses the breeding efficiency of the does. Actually, it is known that, unlike sheep, goats appear relatively intolerant to wet weather (Steel, 1996), but how rain directly affects the reproductive physiology of the doe is not known, though it may be because of chill from cold stress. Griffiths *et al.* (1970) subjected Blackface ewes to cold stress 18 days before mating and reported that ovulation rate was significantly lower in the treatment than control groups (1.52 v 1.86). They concluded that exposure to cold and wet weather at sometime during the estrous cycle prior to mating can reduce fecundity (total potential number of ova that can be shed or progeny that can be born in a breeding interval).

On the other hand, high temperature may affect prolificacy through thermal stress. Kinne (2000), reporting on Pigmy does in USA states that does have lower conception rates in Spring and Summer when both photoperiod and temperature are rising, and that temperature over 90^0F (32^0C) may cause thermal stress especially when combined with high humidity, increased activity and excess body fat (body condition). The depressive effect of high temperature on the sexual behavior and mating activities of both female and male small ruminants has been reported as a source of fertilization failures as well as embryo mortality elsewhere in the tropics (Braden and Mattner, 1970; Kelly, 1986), but not for SSA (Abassa, 1995).

Mellado and Meza-Herrera (2002) investigated the effect of temperature at mating on prolificacy of Mexican does. When the maximum ambient temperature at mating was more than 36^0C, prolificacy of does exposed to higher or lower temperatures one day before mating was 1.56 and 1.65, respectively ($P<0.05$). Similarly when the maximum ambient temperature at mating was 34^0C–36^0C, prolificacy was higher for does exposed to lower temperatures as compared to higher temperatures one day (1.64 v 1.49; $P<0.01$) or 3 days (1.63 v 1.48; $P<0.01$) after mating, with respect to the temperature on the day of mating. They concluded that increment in litter size is expected with lower temperatures before or after hot days at time of mating.

Since the observations were made at the mating (estrus) stage of the breeding cycle it may mean that an excessive rise in ambient temperature (in this case beyond 34^0C-36^0C) for 1-3 days before or after breeding may reduce prolificacy value by inducing pre-embryo losses with thermal stress. According to Hafez (1993), fertilization (which together with cleavage is completed after 1.5 to 2 days in sheep) is lowest in does exposed to elevated ambient temperature prior to breeding. Citing Edey (1969), Abassa (1995), states that field observations show that early embryonic deaths do occur in hot environment; and that sheep embryo is highly susceptible to continuously applied maternal heat stress during early cleavage stages while it is in the fallopian tube, that is, during the first 3 days after ovulation and mating. He continues with the report of Thwaites (1967b; 1967c) that although acclimatization and diurnal variations in temperature can modify the adverse effects of such heat stress, all embryos will die when continuous exposure to high temperature raises ewe rectal temperature by 0.76 to 1.13^0C. In a study similar to that of Mellado and Meza-Herrera (2002), Lindsey *et al.* (1975) showed that when ambient field temperatures were between 32°C and 40°C there were negative correlations between these temperatures at the time of mating and lambing performance, a situation ascribed to embryo mortality. As a result, Abassa (1995) expects strongly that since prolonged heat stress due to high ambient temperatures within or above the range 32°C–40°C prevails during dry season in many areas of SSA – where housing facilities are lacking – it is likely that embryo death may be more than a minor component of reproductive losses in the region.

Another means by which heat stress can cause reduction in fertilization rate is through mating failure resulting from non-exhibition of estrus and libido by the doe and buck respectively. Studies on fertility of Peul ewes in the semi-arid zone of Niger showed that during the period of increasing temperature, silent ovulations were frequent and complete anestrus common (Yenikoye, 1984). In this case, the studies revealed that native ewes and does did not exhibit estrus in spite of the evidence that they had ovulated. Behavioral estrus was shown and reproductive activity was maximal only during parts of the year.

These observations led Abassa (1995) to conclude that temperature may depress reproductive performance in ways such as reduction in the proportion of ewes in estrus throughout the year (Guerra *et al.*, 1972), an increase in the anestrus period (Haresign, 1981) and a failure to mate due to absence of behavioral estrus. This may affect unsheltered animals commonly seen under village conditions, and particularly in Sahelian and arid zones of SSA.

Armstrong (1986) cited by Hafez (1993), explaining how thermal stress may tell on the reproductive capacity of a doe, made two suggestions. He suggested that heat, like other environmental stresses, may interfere with the hypothalamohypophyseal system, resulting in disruption of the normal pattern of gonadotropin secretion; or may alter ovarian function directly or indirectly through other organs, involving metabolic interactions, which change the balance of feedback control of the hypothalamo-pituitary-ovarian system. This may affect follicular development and ovulation.

In the case of fertilization and early embryo development, Hafez (1993) observed that eggs of sheep and cattle when subjected to high temperature either *in vivo* or *in vitro*, were damaged but continue to develop only to die during the critical stages of implantation. Hafez (1993), points out also that the effect of heat stress on embryo can be observed following exposure of a doe to elevated ambient temperatures; and especially in tropical areas, embryo mortality increases. According to Hafez (1993), although the increased maternal temperature directly affects the embryo in early stages of development, only in later stages does it become apparent. Explaining, Monty and Racowsky (1987) cited by Hafez (1993), state that thermal stress may result in decreased viability and developmental capacity of 6-8-day old embryos (especially in dairy cows) or in altering the uterine environment as well as growth and secretory activity of the conceptus between days 8 and 17 (Geisert *et al.*, 1988, cited by Hafez, 1993).

Since the buck supplies the male gamete, which necessarily complements in a way to determine a doe’s prolificacy, mention of the effect of climate on the buck’s reproductive performance cannot be out of place. Abassa (1995) takes note from Waites and Setchell (1969), Braden and Mattner (1970), and Smith (1971) that ram fertility (ability to produce active sperm that can normally fertilize ova) is affected most severely 2-3 weeks later after it is exposed to high ambient temperatures. This lends credence to the explanation of Hafez (1993) that elevated body temperatures, during periods of high ambient temperature or pyrexia (abnormal rise in body temperature) from disease lead to testicular degeneration and reduce the percentage of normal and fertile spermatozoa in the ejaculate. Hafez (1993) explains, more specifically, that when the scrotal contents of rams are heated to approximately 40^{o}C for 1.5 to 2 hours, a sharp increase in the proportion of morphologically abnormal spermatozoa occurs in the ejaculate 14 to 16 days later. Spermatozoa that are developing in the testis at the time of heating show damage (e.g. dead and tailless sperm), whereas epididymal sperm are unaffected. Acrosomal damage is characterized by swelling, vesiculation and eventual disintegration.

According to Abassa (1995), research on the subject is not only neglected but the few available reports (Wilson, 1976; Reynolds, 1979) claimed no adverse effects of temperature on male libido. Wilson (1976) cited by Abassa (1995), working in Southern Darfur in Sudan reported that ram libido appeared to be high at all times. Even though Abassa (1995) doubts the notion of “no thermal stress effect on male libido”, it is apparently re-echoed by inference from the statement of Hafez (1995) that rams may retain a satisfactory level of fertility throughout the whole year, but in many instances, *fertility* (production of viable sperm and not libido per se) is depressed when mating occurs during the hot months of the year; and that conception failure in ewes mated to heat stressed rams is related more to failure of fertilization than to embryo mortality. Kinne (2000) noted that heat stress can cause temporary infertility in bucks, which usually resolves within six weeks after the episode; taking that long for new sperm to develop. This can greatly lower reproductive rate in the tropics especially SSA where there is widespread nutritional and housing constraints prevailing and temperature depresses the female reproductive activity (Abassa, 1995).

The climatic elements that have been particularly observed to have effect on prolificacy are light, rainfall and temperature (Edey, 1969; Rutagwenda *et al.*, 1985; Mellado and Meza-Harrera, 2002), while the important ecological factors for the promotion of normal reproduction are light, warmth and adequate diet (Arthur, 1975). Light tends to influence the timely ovulation in female animals with seasonal estrus (Edey, 1969). Rainfall acts through its influence on variations in feed availability during the breeding interval (Valencia *et al.*, 1990) and therefore may be associated with the complex physiologic mechanisms by which nutrient metabolism affects production of ova and progeny. For temperature, its effects appear to be more significant at the extreme ends where it may act through either cold or heat stresses to adversely affect the survivability of ova and or conceptus during the breeding interval. Although the exact physiologic mechanisms by which these climatic elements affect female reproduction are not well understood it is believed that they eventually send 'signals' that alter either the pattern of gonadotropin secretion by the hypothalamo-pituitary systems or the ovarian functions that maintain a balance feedback control of hypothalamo-pituitary-ovarian system (Armstrong, 1986, cited by Hafez, 1993). Thermal stress in particular may directly render conceptus unviable or alter the uterine environment that supports its viability (Monty and Racowsky, 1987; cited by Hafez, 1993). In regions close to the equator, variations in length of light or photoperiod are not thought to be of sufficient magnitude to entrain the goat's sexual activity. Rather does respond to the other environmental stimuli such as rainy and dry seasons as well as temperature (Valencia *et al.*, 1990).

Table 2.1 THE EFFECTS OF CLIMATIC CONDITIONS AT MATING ON PROLIFICACY OF THE DOE

Country	Source	Type of Climate/season	Characteristics of climate	Reproductive Stage of doe	Effect on doe's prolificacy[1]	Breed of doe
Mozambique	Mckinnon & Rocha (1985)	Wet season (Nov-April)	Hot, Rainy, Forage Growth	Mating (Estrus/ovulatory)	Increase	Landim goats (Tropical)
		Dry season (May-Oct)	Cool, Dry, Poor or no Forage	Mating (Estrus/ovulatory)	Decrease	
Kenya	Rutagwenda et al. (1985)	Wet Seasons, (Mar.-May; Oct.-Nov.)	Hot, Rainy, Forage	Mating (Estrus/ovulatory)	Increase	South East Africa (SEA) goats (Tropical)
		Dry Season (June.-Sept; Dec.-Feb.)	Cool, Dry, Poor Forage	Mating (Estrus/ovulatory)	Decrease	
South Africa	Mamabolo & Webb (2005)	Summer & Spring (Oct.-Jan.) (Aug.-Sept.)	Hot, Rainy, Forage	Mating (Estrus/ovulatory)	Increase	Native goats (Tropical)
		Winter & Autumn (May -July.) ,(Feb.-Apr.)	Cool, Dry Reduced Forage	Mating (Estrus/ovulatory)	Decrease	
Mexico	Mellado & Meza-Herrera (2002)	Spring & Autumn (Jan.- Mar.) (July.-Sept.)	Warm, Rainy forage	Conception (fertilization & Cleavage)	Increase	Nubian Granadino Alpine Toggenburg (Tropical)
		Winter & Summer (Oct.-Dec) (Apr. –Jun.)	Cool, Dry, Reduced forage	Conception	Decrease	
Mexico	Melldo & Meza-Herrera (2002)-	Rainy Weather	Rainfall on day of	Mating	Decrease	Nubian, Granadino, Alpine, Tggenburg. (Tropical-
		Cool, Dry weather	No rainfall; Temp. ≤ 34^{0}C	Mating	Increase	
		Hot weather	No rainfall; Temp. ≤ 36^{0}C	1-3 days after Mating	Decrease	
USA	Kinne (2000)	Summer & Spring (Apr. –Jun.) (Jan. – Mar)	Warm, forage grows.	Conception	Lower	Pigmy (Temperate).
		Fall & Winter (Jul. – Sept.) (Oct. –Dec.)	Cold, reduced forage	Conception	Higher	
		Hot weather	Temperature of day > 32^{0}C	Conception	Lower	

1. Generally, for increasing prolificacy, tropical breeds tend to prefer wet seasons at mating. Perhaps it is because of feed availability at various stages of the doe's reproductive cycle rather than the season *per se*. In addition rainy season's association with availability of feed at mating may result in positive energy balance to offset the effects of cold stress (chill) on prolificacy. Moreover, excessive ambient temperatures beyond 34-36^{0}C before or after mating, which occur during dry season, may promote fertilization failure, and hence lower prolificacy.

2. 4. 3 Effects of Dam Age, Parity and Litter Size

In the doe recurring estrous cycles are associated with shedding of one or more ova (Authur, 1975). Although primate menopause is not observed in domestic female animals, (IAEA, 1984), they eventually become too old to breed efficiently (Peacock, 1996). This indicates that the reproductive efficiency of the doe, in terms of *prolificacy*, determined by *ovulation rate* and *prenatal wastage*, is affected by her age.

Ovulation rate in the doe increases with maturity and remains at an optimum level for a few years until it begins to decline (Peacock, 1996; Kinne 2000). Devendra and McLeroy (1992) report of Kilis does from Turkey and Angora does of Texas, reaching maximum fertility (ability to produce fertilizable ova more readily) at 5 and 7 years of age respectively. In sheep, Findlay and Vaughan (1964) found ovulation rates of 1.21, 1.61 and 1.46 in age groups 1-2, 3-4, and 5-7 years respectively. They concluded that the number of ova shed at each estrus reaches a maximum at 3-4 years of age and falls after 5-6 years. The subsequent number of lambs born per conception was also observed to depend on age (Gordon, 1972). Amoah and Gelaye (1990) reported that litter size at birth of does in South Pacific countries was related significantly to age. In a goat survey, the Manitoba Department of Agriculture, Food and Rural Initiative (2002) observed litter size at birth of 1.84 and 1.70 for mature does and replacement doelings respectively. They attributed the difference to factors such as weight, age and sexual maturity.

Apart from age *per se*, parity and litter size may also affect prolificacy. Jollans (1960) observed in Djallonke sheep that twinning rate increased from 19% during first lambing to 66% during the third, with the latter rate for ewes that were over 5 years old. In a study of Kacang and Peranakan Etawah goats under village production systems in Indonesia Sodiq *et al*. (2003) reported that the prolificacy of these two breeds tended to increase with advancing parity and litter size of pregnancy up to the fourth parity and slightly decreased thereafter. They found their results to be consistent with those of Das (1993) who, working on meat goats in Malya, Tanzania, observed that prolificacy tended to increase from first parity and decreased in the sixth parity. Reporting on twinning rate of Landim goats and Ngungi sheep of Mozambique, McKinnon and Rocha (1985) concluded that sheep tended to have maximum twinning at the fifth parturition, while for goats the maximum twinning percentage was achieved at the third parturition. This corresponds with the observation on Malabar goats for which Devendra and McLeroy (1992) state that the proportion of twins and triplet births increased from 19 percent in the first kidding to 79 percent in the second and later kiddings.

The prolificacy values at the various ages and parities depend also on the rate of prenatal wastage, which is in turn also influenced by dam age and parity. Dolling and Nicholar (1967) had results indicating reduced prolificacy with age of ewes, while Knight *et al*. (1975) found that wastage was not influenced by age of ewes. Other authors agree that age and parity play a part in prenatal losses, beginning with the ovum and spermatozoon. Kinne (2000) found that abnormal development and reduced viability of the egg (resulting in early embryo losses before day 16), increase with age of the doe; and that the older does had a higher incidence of embryonic mortality due to poor egg quality and a less responsive uterus than younger ones. Similarly in sheep, gimmers and old ewes over 6 years lose more embryos than mature (4-5yr) ewes; but this is due to factors associated with the embryo rather than the uterine environment (Hafez, 1993).

In ewes embryonic mortality decreases with increasing parity up to the fifth pregnancy and then begins to increase (Hafez, 1993); and in pigs a higher incidence of embryonic mortality is observed in gilts (yearlings) and in sows after the fifth gestation (Hafez, 1993). In the humid zone of La Cote d'Ivoire, Rombaut and van Vlaenderen (1976) observed that nulliparous ewe lambs bred between four and eight months of age not only suffered from higher abortions and stillbirths (55 percent) than adult multiparous ewes (14.6 percent), but also lost 89 percent of their lambs before one year of age. They strongly recommended that immature females be protected from early contact with mature males in the traditional system where breeding is not controlled.

There are indications also that litter size or uterine biomass of conceptus (Akusu and Ajala, 2000) tends to increase prenatal wastage, especially through abortion and stillbirths. For female caprines in SSA, high rates of abortion and stillbirth were found among twin or triplet bearers (Suleiman, 1976; Osuagwuh, 1984), females with short parturition intervals (Rombaut and van Vlaenderen, 1976) and when primiparous and immature females were bred (Ojo, 1980). Hafez (1993) states that in cattle, horses, and sheep the frequency of multiple pregnancies is higher than that of multiple births. This suggests that high incidence of spontaneous abortion and fetal resorptions are associated with multiple pregnancies. In the mare over two-thirds of twin pregnancies end in abortion. Similarly, in prolific breeds of sheep late embryonic deaths occur more in ewes with more than five ovulations.

When computed in percentages, prolificacy, resulting from the counteracting effects of ovulation rate and prenatal wastage either demonstrate a curvilinear or near parabolic relationship with age/parity of the female animal. In sheep, Hanrahan (1976) reported that prolificacy increased from 105% for one-year-old gimmers to 153% for the 4-year olds. With ewes older than 4 years, there was a slight decline. Oppong Anane (1971) observed a twinning rate of 9.8% for Djallonke gimmers, with a trend of increasing rate up to 3 years of the dam. Baah (1980), analyzing records on the same Djallonke breed and from the same source, showed a general pattern of steady rise with a positive linear regression between age and twinning rate ($r^2 = 0.59$).

There is evidence that age may affect the fertility of the male animal also. According to Hafez (1993) fertilization failure due to mating failure may result from the fact that old bulls, even with high libido, suffer flaccidity of the penis and so are unable to gain intromission during coitus. This condition, which is more common with aged bulls, may be caused by trauma of the tunica albuginea (Hafez, 1993). Furthermore, abnormal development and reduced viability of sperm leading to poor fertilization and early embryo loss increase with age of the male animal (Kinne, 2000).

Prolificacy is controlled, essentially, by reproductive hormones, especially gonadotropins and steroids (Spearow, 1999). The rate of ovulation, which sets the upper limit of prolificacy (Gunn and Doney, 1971), is related to the concentration of FSH (Kinne, 2000) and a timely interplay with LH and steroids (Swenson, 1970). The lower ovulation rate in young and aged does may be due to high incidence of atresia resulting from low FSH and estradiol secretion. Hafez (1993) indicates that low FSH during follicular phase of estrus leads to high atresia rates, and depends on factors including age of the doe. Low FSH causes high atresia because of low stimulation for secretion of estradiol with subsequent delay in granulosa cell growth.

In cattle, it is believed that the higher incidence of embryonic mortality in old cows may be due to a defective uterine environment (Hafez, 1993). Albihn *et al*. (1989) cited by Hafez (1993), showed evidence suggesting that defective environment may be a reason why the early development of bovine embryos is impaired in the uterine.

Steel (1996) speculates that one reason older goats may reduce productivity is because their teeth are often worn out; and with difficulty in mastication they eat less, lose body condition and therefore may not produce multiple ova; or may become more prone to abortifacient diseases.

In the case of litter size, the increasing prenatal wastage with multiple pregnancy may be attributed to overcrowding or competition for placental space in the uterus (Hafez 1993), since placental development is primarily influenced by the availability of space and vascular supply within the uterus. Increasing the number of implantations decreases the vascular supply to each site and restricts placental development. This may be attributed to insufficient supply of nutrients or metabolites from the maternal circulatory system. Hafez (1993) observes in non-polytocous animals such as cattle and sheep that, when multiple ovulation occurs embryonic mortality increases till surviving fertilized ova are reduced to a fairly constant number (2½ to 3 embryos per female) within the first 3 or 4 weeks of pregnancy. He believes that such mortalities do not seem to be due to a deficiency of progesterone.

The lower ovulation rate in nulliparous and primiparous does may also be due to relatively lower number of follicles recruited during folliculogenesis in the preliminary estrous cycles. In ewes, about 20-30 hours after ovulatory surge of LH and FSH, there is a second peak of FSH, which stimulates the recruitment of antral follicles, some of which may be ovulated at the next estrus. Hafez (1993) states that this postovulatory transient peak of FSH that recruits follicles is very high in those ewes with higher ovulation rate; and that the magnitude of the peak is highly correlated with the number of large antral follicles in the ovary at the next estrus. Perhaps in the young doe the rate of secretion of reproductive hormones, especially

FSH, is relatively low and increases with age and parity as the endocrine glands mature and become more functional. But the increase in FSH reaches optimal levels at a mature breeder stage, where the correspondingly rising ovulation rate levels off, possibly being the genetically determined maximum for the species (Kinne, 2000).

One reason embryo mortality is high in aged does may be that the speed of moving early embryo to site of implantation in the uterus is irregular. This may be due to estrogen-progesterone (E_2/P_4 ratio) imbalance culminating in improperly timed arrival of embryo in uterus, poor or no maternal recognition and, hence, early embryonic death. According to Hafez (1993), either accelerated or delayed transport of the egg, resulting from E_2/P_4 imbalance leads to preimplantation death of embryos. Estrogen and progesterone act antagonistically on the motility of the reproductive tract; with P_4 slowing down while E_2 speeding it up (Hansel and McEntee, 1970). A balanced ratio of E_2/P_4 is then required for a timely transport and survival of the fertilized egg into the uterus. Hafez (1993), states that it is critical for a fertilized egg to reach the uterus at an appropriate gestational stage of the cycle. In sheep and cattle this critical time begins between 3 and 4 days to day 12 or 13 after mated ovulation, when E_2/P_4 ratio is low due to rising P_4 from the corpus luteum (CL). However, in aged animals, function of the CL declines as a result of (a) an inability of the follicular cells to respond fully to hormonal stimuli, (b) changes in the quantity or quality of hormone secretion and (c) reduced stimulus for hormone secretion (Hafez, 1993). Hafez (1993) believes that for selective acceptance of blastocyst uterine differentiation occurs upto a critical stage before implantation can take place.

2. 4. 4 Effects of Nutrition

Nutrition is one major non-genetic factor considered to significantly influence reproductive performance in many mammals and poor nutrition is a major constraint to increased ruminant productivity in SSA (Abassa, 1995). The availability of nutrients is the ultimate regulator of reproductive function and severe under nutrition has been reported to lead to cessation of all reproductive activities regardless of other factors (Walkden-Brown, 2001). In fact, the effects of nutrition on reproduction have been recognized for a long time. Medvei (1982) cited in Lucy (2003), mentions Aristotle (384-322 BC) – a Greek philosopher - who wrote that nutrition was the most important environmental factor controlling conception. Since then studies on the effects of nutrition on female reproductive performance have shown similar results (Lucy, 2003). Deficiency of the trace minerals copper, iodine, magnesium and selenium are reported to interfere with conception, in addition to protein and energy (Kinne, 2000). Pregnancy in vitamin–A deficient ewes usually terminates in abortion or birth of either weak or dead lambs (Assoku, 1972).

Walkden–Brown (2001) observed that nutrition influences the timing of the pubertal age and in cyclic does seasonal and post partum transitions to reproductive competence. It potentiates ovulation rate, sperm production and sexual behavior in reproductive mature adults. Hafez (1993) also noted that the blastocyst and early embryo are nourished by endometrial fluid. The fetus also receives its supply of nutrients from the maternal circulation across the placenta. Severe under nutrition during pregnancy may result in a loss of conceptus and/or mother, because of maternal nutritional stress; or it may lead to retarded growth and impair development of the fetus. In caprines under nutrition during late pregnancy leads to stunted progeny, even when a normal level of nutrition was present earlier (Hafez, 1993).

It appears that nutrition clearly impacts several aspects of reproduction (Hileman *et al.,* 2000). According to Foote *et al.* (1970) cited in Valencia *et al*. (1990), the three main phases of the caprine female reproductive life-cycle are: the prenatal and perinatal (or early neonatal) preparatory phase, pubertal adjustment phase, and cyclic ovarian activity phase.

2.4.4-1 The Prenatal (gestation) Phase Nutritional Effects

During the early or pre-implantation part of prenatal phase the uterine fluid, which is secreted by the endometrial epithelium and provides a favorable environment for sperm capacitation, provides also nutrition for the blastocyst until implantation (Hafez, 1993). In sheep the volume of the fluid in the uterus exceeds that of the oviduct during estrus, whereas during the luteal phase, the reverse is true (Hafez, 1993). This indicates that the nutritional function of the uterine fluid begins earlier with fertilization and cleavage of the zygote; and is altered at various locations to ensure adequate nourishment of the conceptus as it moves from the fertilization site in the oviduct towards site of implantation in the uterus. In the cow, Hafez (1993) observes that the embryo lies in the medium for approximately 30 days, during which time extensive embryonic differentiation takes place before the conceptus becomes firmly attached to the endometrium. It, therefore, will imply that any poverty in the nutritional capacity of the uterine fluid may adversely affect embryonic gonadogenesis (initiation of gonadal tissue differentiation) and formation of accessory reproductive organs.

After implantation, the embryo depends on the vascular supply within the endometrium for its development (Hafez, 1993). The practical implication is that during the post-implantation stage of the prenatal life the nutritional status of the mother is critical since the fetus receives its supply of nutrition directly from the maternal circulation across the placenta, until parturition; and hence, the need for nutritional attention to the expectant dam at this time. Hafez (1993) infers that the physiologic properties of the uterine endometrium and the supplies (of nutrients) in its blood throughout gestation are important for the survival and development of the fetus.

Nutritional supply during gestation is important because it affects the prolificacy of the mother through influence on the survivability of its fetus; and it may affect the future reproductive capacity of the fetus by influencing the development of its rudimentary reproductive organs and functions (Foote *et al* (1970, cited in Valencia *et al.*, 1990; Hafez, 1993). It is believed that nutritional factors have both short and long term effects on growth, body composition and function (including neural and gonadal functions). In cows supplemented heifers had larger ovaries and attained puberty earlier than the unsuplemented (Mukasa–Mugerwa, 1989) which suggests that it is possible for gonads to under-develop due to nutritional stress. For this reason and to avoid any adverse effects on reproduction, of both fetus and mother, the practice of steaming-up or late pregnancy feeding in ruminants evolved (Robinson, 1974). Late pregnancy feeding, therefore, aims at the growth, development (including rudimentary reproductive system) and survival of the fetus, as well as to provide a good body condition for the mother against parturition and lactation stresses.

The growth and development of the fetus, parturition and involution of the uterus, all use energy; and this energy must be supplied to the pregnant animal if she is to rebreed soon. Blasu (1981) subjected 45 Djallonke ewes to supplementation treatment for the last six weeks of pregnancy in Ghana. The supplemented ewes gained a mean of 3.6kg /ewe ($P<0.05$) more, and had heavier and thriftier lambs with higher perinatal survival than the controls. ILCA (1987b) reports on a 2-year browse supplementation trial at Ibadan, Nigeria. It was found that sheep and goats offered leucaena and gliricidia for the last 2 months of pregnancy and during lactation, as supplements to a basal diet of *Panicum maximum*, had survival and growth rates of their offspring increased with the level of supplementation. In another report, pregnant cows fed 3.5kg of cowpea daily, as supplement to basal diet of bush hay, for 3 months in Kala Nmapala, Mali, had improved body condition, increased birth weight by 27% and increased total milk production by 17% (ILCA, 1987c).

Since the early twentieth century some explanations have been given to the observed effects of late gestation feeding on both fetus and mother. Thompson and Aitken (1957) showed that 70% of the final weight of the fetus was laid down in the last 40-50 days of pregnancy in sheep. Nalbandov (1976) explains that this rapid growth in late gestation is possible because the fetus, with its more efficient physiological system, diverts so much nutrient material from the maternal circulation to its own use at the expense of the mother. Wallace (1948) cited in Thompson and Aitken (1957), indicated that towards the end of gestation it is the ration being fed rather than the state of the maternal reserves that limits the growth of the gravid uterus. In cows, Mukasa-Mugerwa (1989) suggests that although the pregnant cow can meet some of its energy requirements from body reserves or fat, the farmer must supply the bulk of the needs, especially in late gestation. Domfeh (1972) and Banerjee (1976) have suggested that pregnant caprine females be supplemented for their additional energy requirements during the last 8 or 6 weeks of gestation. Benerjee (1976) suggested that this supplementation could be increased by as much as 50% of the maintenance level. However, Kinne (2000), reporting on Pigmy goats, warned that overfeeding and lack of exercise lead to dystocia and that over-feeding single-bearing does in the last weeks of gestation increased birth weight of the fetus by 2.2% compared to does not overfed. In cattle, Mukasa-Mugerwa (1989) suggested that ideally the cow’s body condition should improve gradually through pregnancy, and that excessive fatness prior to calving should be avoided.

A number of studies have shown a decrease in embryo survival due to nutritional restriction (Bennet *et al.,* 1964; Edey, 1966 and 1970a; Cumming, 1972a and 1972b; Blockey *et al.,* 1974), though others have reported no such effects (Killeen, 1967; Bennet *et al*., 1970; Smith *et al.,* 1983 – all cited in Abassa, 1995). Blockey *et al.* (1974) cited in Abassa (1995), investigating the suggestion of van Niekerk *et al.,* (1968) that a few days of fasting could kill embryos, found that three days of fasting killed up to 10 percent of single embryos during the first ten days after mating but had no effect on twin embryos. The authors concluded that there would normally be little effect on lambing percentage since deaths occurred early and ewes returning to service could mate again. Kelly (1984) cited by Abassa (1995) concluded that under normal farming conditions, it is unlikely that such severe changes in nutrition will occur.

Apparently, nutritional requirements during pregnancy are not only met by food intake during this period, but also by nutrient supply before or between pregnancies (Veldhuis *et al.*, 2005). Most of the reports and views on the issue do not mention nor seem to consider any residual effects of the metabolic state of the dam at the time of breeding. Yet Hafez (1993) states that the caloric intake and specific nutritional deficiencies of the dam at mating affect not only ovulation and fertilization rates, but also cause prenatal death. He observes that, in swine, high caloric intake or continuous unlimited feeding increases ovulation rate, thereby increasing the incidence of embryonic mortality before implantation. Poor body condition of ewes at mating increases the incidence of embryonic mortality, whereas moderate feed restriction from day 20 to 100 of pregnancy is less likely to reduce lambing percentages (Hafez, 1993). Mapletoft *et al.,* (1986) cited by Lucy (2003), reported that studies involving embryo transfer showed that recipients with low BCS had lower conception rates when compared to recipients with high BCS. This implies that a poor maternal nutritional status may cause even a healthy embryo to lose viability; and this viability loss may have occurred earlier and carried-over from ovulation/mating time. Fogwell (1997) explains that follicles that develop during negative energy balance (NEB) are often too small to achieve dominance and/or fertilization competence.

It may be suggested that both high and low extremes of the nutritional status of the dam at mating may residually induce mortality of the early embryo before implantation. This may happen through the effects of nutrition on the secretory preparations of the ovary and uterus at the time of breeding. The early embryo is solely nourished by the uterine fluid, secreted by the endometrium upon stimulation by preovulatory estradiol; and its growth and implantation is also controlled by this fluid (Hafez, 1993). Since preovulatory estradiol is secreted mainly by the preovulatory follicle, and since poor caloric intake of dam at mating reduces the ovulation rate, the level of estradiol stimulation of the endometrium and, subsequent response in secreting a nourishing uterine fluid to support growth of the fertilized egg may be reduced in the dam with poor body condition at mating. Furthermore, where the dam's nutritional status at mating is in excess, the endometrial cells may be exposed to more starting materials (nutrients) for increased metabolism and perhaps synthesize more uterine-specific proteins that may be immunologically agonistic to normal embryonic growth and implantation. Specific proteins of uterine or conceptus origin have been identified and characterized (and purified) during early pregnancy in the ewe; however it is not clear whether they can influence cell mediated immune responses (Hafez,1993).

About 75% of the reported abortions in goats in Niger occurred towards the end of the dry season (Abassa, 1995). In Burkina Faso, 21.6% of abortions took place in the rainy season, 29.9% in the dry cool season and 48.5% in the dry hot season. It was estimated that 23.6 to 87.5 percent of breeding ewes and 27.3 to 72.2 percent breeding does joined in the dry hot season lost their fetuses before term. These are indications that season can adversely affect reproduction, especially because of scarcity of feed resources during the dry season (Rutagwenda *et al.*, 1985). The latter authors obtained 22 and 0 percent abortion rates when females were mated at the end of long dry season and prior to the long rains, respectively. In the humid zone of La Cote d'Ivoire, the detrimental effect of season seems to be more important in the first months (April-May) of the long rainy season where more than 50 percent of breeding females lost their fetuses before term (Ambruster, 1980, cited by Abassa, 1995).

Apart from nutritional adverse effects through season, management practices found in crop producing areas of the humid and sub-humid zones are also thought to be responsible for nutritionally induced fetal losses. In these zones, small ruminants are tethered during rainy seasons to prevent them from damaging crops (Mack *et al.*, 1984). This leads to undernourishment, which results in weight and body condition losses with poor reproductive performance occurring, ironically, during the very period when feed resources are abundant (Abassa, 1995).

The period of high vulnerability when nutritionally induced abortions occurred in goats was found to be between 90 and 100 days of fetal life (Shelton and Groft, 1974). This was confirmed by Osuagwuh and Akpokodjie (1985) who found that abortions are the prominent responses of WAD does to undernourishment and that most of these abortions occurred during the period of accelerated fetal growth i.e. 90-120 days of fetal life (Abassa, 1995).

Due to metabolic programming – a condition whereby nutrients consumed during the first weeks and months of life may have permanent effects on adult metabolism – a poor nutrition in very early life is already a poor starter or foundation for general nutrition-dependent physiologic development in the future, including reproduction. For all these developmental and maintenance processes the embryo and fetus require and obtains food substrate such as glucose, amino acids, vitamins and minerals from the maternal supplies in placental blood and uterine fluid (Hafez, 1993).

The fetus absorbs and metabolizes the substrates within its cells for energy and growth – through protein synthesis and cell division. Generally, these metabolic activities result in formation of numerous oogonia (germ cells for future ova), which develop into a fixed number of primordial follicles at birth (in the doe). At the same time elaboration and secretion of LH, FSH and LH-RH also begin within the first 4-8 weeks of pregnancy (Hafez, 1993), but secretion reduces temporarily by one month before kidding. The rate of the metabolism depends on the availability of the food substrates and the genetic ability of the fetus to use them (Hafez, 1993). Owen (1991) cited in Hafez (1993), expresses that insulin and insulin-like growth factors (IGF-I and -II), which occur in fetal and placental tissues, mediate the availability of energy, and participate in regulating the metabolic activities of the mother, so that a continuous supply of feed substrates for fetal development are always available. In addition the fetus has a unique ability to deplete maternal skeletal stores of calcium if feeds are low in calcium (Hafez, 1993). This explains why it may be necessary to consider inclusion of a calcium source in gestational rations.

2.4.4-2 Pubertal Phase Nutritional Effects

As stated earlier, the miniature or rudimentary female reproductive gonads (ovaries with fixed amount of primordial follicles) and gonadotropic functions (LH and FSH secretions) developed by the doe-kid as at early neonatal stage, have to mature to adult profiles before normal reproductive cyclicity can occur (Hafez, 1993). Arthur (1975) noted that the female animal is born with a genetic potential for cyclic reproductive activity, the realization of which appears to depend on an age-maturity of the hypothalamus and mid-brain sex centers. At the time or age when this realization first occurs – called puberty – a “biological clock’ is started and continues for as long as the environment remains favorable.

Puberty is the age at which reproductive organs begin their cyclic physiological function for the first time, after the prenatal preparatory development (Frandson, 1974). At this stage the doe-kid begins to ovulate and manifest complete sexual behavior (Lavasseur and Thibault, 1980, cited in Valencia *et al.*, 1990). Puberty is a function of genetics and age-maturity (Arthur, 1975), but the latter is itself influenced by environmental factors (Hafez, 1993), the most important of which are photoperiod, temperature, and body weight as affected by nutrition (Hafez, 1993) as well as socio-sexual effects (Gelez and Fabre-Nys, 2004). In the tropics nutrition is the most important of the major factors that affect the onset of sexual maturity (IAEA, 1989; Devendra and McLeroy, 1992).

Mukasa-Mugerwa (1989) in his review concluded that, for cattle, poor nutrition delays sexual maturity reduces conception rate and increases pregnancy losses in heifers. He cited Post and Reich (1980) who reported that drought delayed the onset of puberty in heifers of 10 breeds in Australia and stopped ovarian activity in half of those that had already reached puberty; and also Bartha (1971), who found that feeding concentrates to Azaouak zebu heifers advanced puberty and first conception by 4 to 18 months. Hafez (1993) and Wildeus (1996) observed that a low plane of nutrition delays first estrus and reduces body, uterine and ovarian weights; while raising the plane of nutrition increases growth rate before and after weaning and advances the onset of sexual maturity.

However, overfeeding may also be undesirable as it may cause adverse effects. In his review, Mukasa-Mugerwa (1989) cited Penzhoru (1975) who reported on Africander heifers that conception rates, after a 3-month breeding period, were 80, 93, 87 and 40% for heifers on high, medium, low and restricted levels of nutrition, respectively. It was concluded that overfeeding can reduce reproductive performance. Similarly, Reid *et al.,* (1964), cited in Mukasa-Mugerwa (1989), found that heifers reared on a very high level of nutrition had more breeding problems than those fed moderately. According to Wildeus (1996), overfeeding prepubertal goats will decrease subsequent fertility and impair mammary gland development.

Citing authors such as Cameron and Nosbisch (1991) and Foster and Nagatani (1999), Hileman *et al.,* (2000) stated that negative influence of insufficient nutrition on the hypothalamic–pituitary–gonadal axis is primarily realized through reduced pituitary LH release. The observed effects during prepubertal phase indicate that nutrition may affect the determination of puberty by impacting the doe-kid in two ways. First, it impacts the physical development or growth as depicted by size of the body mass; and second, it impacts maturation of the reproductive and neuroendocrine organs in terms of size (or potency) and function (activity).

2.3.4-2.1 Physical development impact

An important early observation was that the timing of puberty is predicted more precisely by body weight than by chronological age (Kennedy and Mitra, 1963). In cattle, Karikari (1990) found that N'Dama heifers (a small breed) in Ghana reached puberty at a mean weight of 172.63 ± 24.5kg, about 64% of mature weight. He cited Williamson and Payne (1978), who had recommended that live weights of 200–225kg for smaller breeds and 290–315kg for larger breeds as appropriate weights for first breeding. In small ruminants, even though published data recognize the greater importance of live weight than age, most reports tend to indicate the latter rather than the former in association with puberty. For instance, goats are said to attain puberty between 5 and 7 months (Hafez, 1963). However, Djallonke ewe-lambs in Western Nigeria reached puberty at 9 months, when they weighed 10.4kg (Devendra and McLeroy, 1992). Peacock (1996) and Wildeus (1996) suggested that female small ruminants should first be bred when they attain 60-70% of adult weight.

Sorensen *et al.,* (1959) conducted a series of experiments to determine the influence of underfeeding and overfeeding on growth and development of Holstein heifers. Apart from body weight they also recorded body measurements consisting of height at withers, body length and heart girth. They found that there was the tendency for heifers to come on estrus for the first time at a given stage of skeletal growth, irrespective of nutrition. They concluded that body measurements at first estrus appear to be less variable than weights, and therefore, more useful in predicting first estrus in a given animal.

That nutrition can affect skeletal formation has been reported. It has been observed in humans and demonstrated in mice that bone formation may be regulated by nutrition through hormonal signals (Takeda *et al.,* 2003). Specifically, leptin (an adipocyte-derived hormone) has been identified as a strong inhibitor of bone formation; its antiosteogenic function being mediated through some hypothalamic neurons different from those regulating energy metabolism, and the sympathetic nervous system peripherally (Takeda *et al.,* 20003). Moreover, in humans, bone mass is maintained constant between osteoblast and osteoclast activity, with that of osteoblast being controlled by leptin (Takeda *et al.,* 2003). If the situation is similar in ruminants it may be suggestive that a critical level of leptin incidence is associated with the skeletal development at puberty.

Frisch (1980) suggested that the level of body fat could in some way trigger initiation of reproductive function. In their contribution to find the link between nutrition and reproduction, McCann and Hansel (1986), investigated the effects of fasting for 8 days on plasma concentrations of metabolites and hormones, in cyclic heifers. They found that concentrations of insulin and glucose were decreased ($P<0.05$) by 12 and 36h, respectively, after fasting was begun and did not return to control values until 12h (insulin) and 4 to 7days (glucose) after fasting ended. Their result agreed with that of earlier workers who found that fasting for 2-4 days decreased concentrations of insulin and glucose in ruminants (Bassett, 1975; Trenkle, 1978 – both cited in McCann and Hansel, 1986).

In a review, Mukasa-Mugerwa (1989) reported that Patil and Deshpande (1981) found that Gir cows that gained weight in the first three months after parturition and showed heat during that period, while those that lost weight remained anestrus (absence of sexual activity, a condition physiologically similar to absence of puberty). Cows that lost weight had lower blood glucose and serum protein concentration than cows that gained weight. One can suggest that blood levels of insulin, glucose and protein are positively related with feed levels and body weight. Maffei *et al*. (1995) and Flier (1998) showed that in the fed state circulating levels of leptin and leptin messenger RNA (mRNA) are correlated with degree of adiposity, and with caloric restriction, leptin levels fall rapidly. All these results put together suggest that rising level of nutrition is associated with gain in body weight, adiposity, blood metabolites and metabolic hormones.

It seems then that the physical impact of nutrition on puberty is by enhancing build-up of the body mass and metabolites as well as metabolic hormones that can support normal sexual activity when it begins, at puberty. It is also believed that in affecting the onset of puberty nutrition appears to be more closely related to overall body composition than to size or weight (Walker *et al*., 1994). Body composition concerns the changes in chemical constitution of the animal body as it grows, resulting in varying proportions of fat and proteins becoming the major materials stored by the animal (Chesworth, 1992; McDonald *et al*., 1998). Building up the body mass involves availing food substrates for the biosynthesis of the body's macromolecules. According to Zubay *et al.* (1995), living cells require a steady supply of starting materials (micromolecules and energy) from intermediary metabolism for the synthesis of macromolecules and other energy requiring processes. The intermediary micro metabolites often needed are amino acids for protein synthesis, fatty acids for lipid synthesis, nucleosidetriophosphate for nucleic acid synthesis and sugars for polysaccharide synthesis. The overall result of these molecular processes will increase cell division and tissue build up, leading to increased body weight, body measurements, fat mass or adiposity, level of metabolites, hormones and growth factors.

Zubay *et al.,* (1995) stated that most often the metabolic state of an organism is reflected by the small molecules present in plasma; thus during prolonged starvation glucose level drops about 30%, while fatty acid level rises about two-fold, and ketone bodies' level rises several fold. They further explained that whereas there is no equilibrium, living organisms do attain a steady metabolic state in the attempt to meet the varying demands for energy and starting materials in their different biological processes. To meet these fluctuating needs, the rate of the reaction sequences in intermediary metabolism must be adjustable over broad ranges. Living organisms adjust these rates so that the concentrations of key metabolites are remarkably stable. This constancy is achieved by maintaining a rate of synthesis for each intermediate that balances its rate of utilization.

Furthermore, in addition to relying on nutrition to supply the chemical fuel (especially glucose) vertebrates maintain fuel reserves in various tissues that make up the body mass (Zubay *et al*., 1995). These reserves are of three types: glycogen, stored within liver and muscle fibers; triacylglycerols, stored in adipose tissues (or adipocytes); and proteins stored as muscle tissue. They further explained that, generally, the small amount of glycogen in the liver, followed by the greater reserves in muscle, is depleted first. This is because glycogen is the easiest of all three types of fuel reserves to be converted into glucose, the immediate useful energy; and only the liver is capable of degrading glycogen to (neutral) glucose that can be secreted into the blood stream. After glycogen come the lipids (triacylglycerols), which contain highest calorific value and are, often in the largest measure of the reserves, in the average individual. Finally the proteins, which make up muscle tissue, are called upon, when the lipid reserve is depleted, and broken-down to amino acids that are transported to the liver for deamination and conversion eventually to glucose.

Body condition as index of nutritional status

The use of muscle tissue proteins to satisfy energy needs is bound to physically weaken the organism; hence its use as the last resort. Perhaps this, together with the view that physical development due to nutrition impacts puberty, through provision of a supportive energy reserve in body mass for reproduction, led to the idea of measuring physical development more as body condition rather than body weight, to determine reproductive readiness. That idea might have gained further support from the fact that fat mass does not only contain the highest calorific value, but also is in the largest measure in the body mass. Impliedly, then, a critical body condition score associated with puberty will represent a critical energy reserve (i.e. positive energy balance) or metabolic status than body weight will do. For, according to van Nierkerk

(1982) body condition score is based on the amount of fat and muscle tissue covering the skeletal frame, and it is indicative of the animal's nutritional status.

In sheep, Oppong-Anane (1975) and Blasu (1981) demonstrated that body condition score (BCS) tended to relate better with prolificacy than live weight. Moreover, the skill is easy to learn and practised with time (Keown, 1996), requiring no assistance of mechanical or electronic devices, unlike body weight, especially in the case of small ruminants. Citing Nicholson and Butterworth (1986), Mukasa-Mugerwa (1989) stated that condition scoring in cattle is a subjective visual assessment of animals, but with practice a high level of repeatability, both between measurements and between scorers, can be obtained. Nix (2004), affirmed the simplicity of the concept, but added that, in goats, just looking at the animal often does not give an accurate measure of the body condition. The system is therefore based on a combination of sight and touching the key indicator areas, which are the back bone, the ribs and the loin.

There are various systems developed for body condition scoring. According to Mukasa-Mugerwa (1989), though the condition score gives a good indication of fatness, breeds differ in the way they deposit fat reserves. This is especially true of cows with more than 15% body fat. Dairy cattle generally deposit more fat internally than do beef cattle. Therefore, at any given condition score value, dairy cows tend to have more fat reserves than beef cows. Similarly in small ruminants, Peacock (1996) stated that goats deposit less fat in the body than sheep. This may be because goats are unable to utilize nutrients as effectively as sheep (ILCA, 1987b). It is possible then that the same body condition score (for example, 3.0) for the two species of same live weight may not be indicative of same metabolic status in terms of quantitative bioenergetics, even though they may have the same qualitative description or interpretation of the score (for example, good). In addition, the energy content of the gains of body tissues can be regarded as varying primarily with age (Chesworth, 1992). The young growing animal has less fat but more protein, while it is vice versa in adults. So the same BCS for kid and doe is not indicative of the same energy content. Different scoring scales, therefore, have arisen for different species in different places.

According to Mukasa-Mugerwa (1989), body condition scoring started in Australia for assessing sheep fatness. The originator, Jefferies (1961) then developed a six-point grading system (hexo-scale) for the concept, from zero to five, corresponding to levels of leanness, with zero as a dying emaciated condition and five as very fat. Over the years other grading systems have been developed in many places for various purposes. There is the five-point system (pento-scale) for sheep (Morrical 1986); goats (Kinne, 2004) and dairy cattle (Keown, 1996). There is a 9-point system (nano-scale) for beef cattle (Lamb, 1999) and for goats – although goats do not normally attain scores 8 and 9 (Luginbuhl, 1998). In all the systems score numbers correspond also to descriptive interpretations that provide a quick qualitative appreciation for the metabolic state measured (Table2.2.), but not calorific values.

Table 2.2 Body Condition Scoring Systems and Score Interpretations for Small Ruminants

	Score Interpretation										
System/ score	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Source**
5-Point system	**-**	**V. Thin or Poor**	**Thin**	**Good**	**Fat**	**V. Fat or obese**	**-**	**-**	**-**	**-**	**Kinne, 2004, USA**
6-Point system	**Emaciated or dying**	**V. Lean**	**Lean**	**Good**	**Fat**	**V. Fat or obese-**	**-**	**-**	**-**	**-**	**Jefferies, 1961 Australia**
9-Point system	**-**	**Thin**	**Thin**	**Thin**	**Moderate**	**Moderate**	**Moderate**	**Fat**	**Fat**	**Fat**	**Luginbuhl, 1998 NCSU**

Therefore, although a condition score is indicative of the animal's nutritional status (van Nierkerk, 1982) it is not an empirical assessment of the actual energy content of the animal. However, in cattle Wright and Russell (1984) cited in Mukasa-Mugerwa (1989), are reported to have established a relationship between the BCS and body fatness in cows slaughtered at different BCS. Thus Wright (1985) cited in Mukasa-Mugerwa 1989) estimated that the loss of one unit in condition score would supply 3,200 MJ of metabolizable energy (ME); while restoring the animal's score would require about 6,500MJ of dietary ME.

This agrees with van Nierkerk (1982) who concluded that the feed cost of maintaining a cow at a condition score of 3.0 was half that required to raise a cow's condition from 1.5 to 3.0. If the same trend occurs in small ruminants then it means that as the doe-kid grows from neonate to puberty, the development of its physical biomass, measured as BCS, not only requires more nutrition, but also is indicative of increasing or positive energy balance. Energy balance (EB) is defined as the net energy that is consumed minus net energy required for maintenance and production (Lamb, 1999). It may be regarded as a form of chemical potential energy that can be supplied either in diet or from body reserves, for the energy dependent biological processes, such as those that trigger puberty at the right time. Hileman *et al* (2000) pointed out some evidences that normal GnRH neuronal activity is dependent on sufficient energy availability, suggesting the synthesis and release of GnRH requires adequate energy. In goats, Ohkura *et al* (2004) have demonstrated in Japan that glucose availability, but not fatty acids, regulates the GnRH pulse generator activity; and concluded that glucose is a key metabolic regulator that fine-tunes pulsatile GnRH release.

Thus, in addition to providing the carbon skeletons (from glucose and fatty acids) and nitrogenous bases (from proteins) dietary macromolecules provide also the energy-potent chemical – adenosine triphosphate (ATP), through intermediary metabolism, for the intracellular biosynthesis and secretion (Zubay *et al*, 1995) of the reproductive hormones in the cells of the hypothalamus-pituitary-ovarian axis. According to Mtimuni (1995), many of the effects of nutrition on reproduction are through influencing the rates of either the anabolism (production) or catabolism (destruction) of gonadotropins.

Energy balance is therefore, probably the single most important nutritional factor (and perhaps followed by protein) that is related to poor reproductive performance, including onset of puberty (Lamb, 1999). According to Mukasa-Mugerwa (1989), although protein is generally regarded as less important than energy for reproduction, low protein intake can also cause infertility, at least, because protein deficiency usually leads to decreased appetite.

Although the energy balance (EB) required for onset of puberty, and the calorific values of body condition scores are not published, it appears BCS between 2 and 3 may be the optimum for puberty in small ruminants, on the 5-point scale. In sheep Morrical and Hettel (1986), suggested that ewes be bred at score 3. For Pigmy goats, Kinne (2004) stated that scores 1-3 represent muscle growth/expansion; and that muscle does not grow after score 3. The last two scores – 4 and 5 – represent fat accumulation and are not desirable for good reproduction. Since live weight and BCS do not seem to give actual values or the concentration of energy and other nutrients in the biomass of the pubertal animal, a closer estimate could be made by direct measurement of these nutrients in the blood, at a steady state. According to Zubay *et al.,* (1995), whereas there is no equilibrium, there is a steady or stable state of nutrient concentration in plasma; and the level of serum total protein can be indication of protein synthetic rate (Tighe and Brown, 2003).

It may be suggested from the bioenergetics of animal nutrition that such a steady state may be attained during basal metabolism. Basal metabolism (BM) is the heat (energy) production during complete rest in a thermoneutral environment in post absorptive (fasting) condition; which in ruminants may be 3 days after last meal. In a thermoneutral environment the animal is not gaining or losing heat (energy). BM is, therefore, the resting energy metabolism in a thermoneutral environment uncomplicated by the heat increment of feeding (Chesworth, 1992). This means that during BM the EB is near zero, and the metabolic system of the animal does not use energy from new dietary source, but from residues of previous meal and small amounts of glycogen from body reserves. It does not also mobilize dietary energy into reserve tissues. Yet at this time basal physiologic processes pertinent to maintenance of life, mainly, blood circulation, respiration and irritability, continue.

Irritability is concerned with the animal's sensitivity or responsiveness to stimulations of both the internal and external environment, which demand co-ordination processes. It therefore involves secretions, including neural and endocrine hormones and factors; muscle tones and maintenance of body temperature. Blood circulation accounts for 5-15% of the total BM energy requirements; another 5-15% goes for respiration and the rest, almost 75%, is needed for irritability (Brody, 1945 cited by McDonald *et al*, 1998).

It follows that the quantum of energy needed immediately (in the short term) for the fundamental physiological processes of reproduction such as gametogenesis as well as the biosynthesis and secretion of reproductive hormones is rather minimal, relative to the full energy requirement of the animal for maintenance and production. According to Lucy (2003), the energy required for ovulation, formation of corpus luteum and maintenance of the early pregnancy is miniscule compared with energy for the latter part of pregnancy and lactation. He, therefore, states that it is theoretically desirous to establish pregnancy of dairy cows in early lactation when seemingly little energy is required for the pregnancy; but this is also the time when, according to Fogwell (1997), energy balance is negative for most dairy cows. Impliedly then, the fundamental reproductive processes like gametogenesis can occur, at least, at basal rates even when EB is at its nadir, supporting the minimal need of energy for these processes. Mtimuni (1995) states that the nutrient requirements for reproduction and lactation are divided into 3 phases: the gametogenetic, gestation and lactation phases. He adds that mammals have small nutrient requirements for the gametogenetic phase, moderate for the gestation and large for the lactation phase.

Peripheral signals of nutrition to the brain

The bioenergetic value of the "small nutrient" requirements for gametogenetic phase, which is the phase that occurs at the onset of puberty or first ovulation post-partum, is not published. Thus a known critical body condition score associated with puberty does not necessarily tell the calorific magnitude of the EB at this stage of the animal's physical development, when there is a mature adjustment of steroidogenesis and gonadotropin secretion (Hafez, 1993). Nor does the pubertal BCS denote the signal(s) of the "small nutritional" status, no matter its calorific value, that inform(s) the hypothalamic-pituitary-ovarian axis about the attainment of the required physical development for reproductive activity. Rather there are numerous peripheral signals reflecting energy stores, recent nutritional state, and other parameters, which are integrated in the central nervous system, particularly in the hypothalamus, to co-ordinate energy intake and expenditure (Havel, 2001; Badman and Flier, 2005). These include short-term (current or postprandal) sensors such as changes in circulating dietary metabolites (glucose, fatty acids, and amino acids) and gastrointestinal (GI) peptide hormones, most notably cholecystokinin (Havel, 2001); and long-term (body reserve) sensors which are usually metabolic hormones and factors, typical of which are leptin and insulin (Havel, 2001; Webb *et al.*, 2004; Ahima, 2005).

In this case, since the energy requirement for the gametogenetic phase of reproduction is minimal (Mtimuni, 1995) and the process may occur even when EB is at its nadir (Lucy, 2003) short-term nutritional signals (like changes in circulating glucose concentrations) may be sufficient to signify the cyclic status of does, as proposed by Patil and Deshpande (1981) in Mukasa-Mugerwa (1989), because GnRH/LH secretion is glucose dependent (Ohkura *et al.,* 2004). However, in predicting puberty, it appears that the integration of the two signaling systems may be ideal, with emphasis on the involvement of either insulin or leptin, among others (Havel, 2001; Fraley, 2004; Ahima, 2004 and 2005; Xu *et al*., 2005; Jose *et al*., 2005; Salvi *et al*., 2006). According to Fraley (2004) insulin and leptin regulate the activity of hypothalamus which is responsible for GnRH that stimulates gonadotropin production by the pituitary; and also have been shown to have influence on follicle function (Webb *et al.*, 2004). The discovery of leptin marked a major advance in the field (Hileman *et al.*, 2000); and there is increasing evidence that apart from insulin, leptin which is produced primarily by adipocytes, may act also as a good signal linking nutritional status with reproductive performance (Spicer, 2001; Ahima, 2004).

Generally, the metabolic hormones are believed to affect reproduction by ensuring the availability of energy for the intracellular metabolism and secretion of reproductive hormones (both gonadotropins and ovarian steroids) and development of ovarian follicles (Webb *et al*, 2004).

In a summary it is established that nutrition, especially decreased availability of metabolic fuels and metabolic hormones, attenuate reproductive activity (Fraley, 2004). The metabolic fuels *per se* may produce sufficient peripheral signals about current or short-term nutritional status; but may have to interact with metabolic hormones for long-term or adiposity signals. The commonest metabolic hormones usually reported are growth hormone (GH), insulin, insulin-like growth factors (IGFs) and leptin. However, the interactions between the reproductive-endocrine and metabolic axes have still not been clearly determined (Cassy *et al*, 2004; Fraley, 2004), although useful insights have emerged from the related studies suggesting either insulin (McCann and Hansel, 1986) or leptin (Ahima, 2004) acting singly or in concert (Fraley, 2004), to be strong candidates for the long-sought link between energy reserves and reproduction.

Apart from its impact on physical development, nutrition affects the onset of puberty also through its influence on the maturation of the ovarian and gonadotropic functions on the prepubertal animal.

2.4.4-2.2 The impact on Physiological Maturation

Alongside its impact on the physical development, nutrition impacts also on the maturation of the reproductive physiology of the pre-pubertal doe-kid. This physiologic maturation, (which ends in the first ovulation as indicative of puberty with or without behavioral estrus), precedes attainment of the adequate physical status for normal reproductive activity. Hence, the suggestion to delay first breeding till the growing doe-kid attains 60 – 70 % of adult body weight (Steel, 1996), and when the kid would have passed the period of adolescent sterility (Hafez, 1993).

The physiologic maturation involves the enlargement (i.e. development of potency or capability) of the reproductive organs, and a gradual increase in their secretory abilities. The physiologic functions of importance, as puberty approaches, are the ovarian adjustment of gametogenesis and steroidogenesis; and the pituitary's expression and secretion of gonadotropins (Hafez, 1993). Arthur (1975) deduced that puberty depends on the initiation of pituitary release of gonadotropin and that it must therefore correspond with maturation of the hypothalamus. One can, therefore, suggest that the maturation of the hypothalamus is paramount in onset of puberty, and that the rate of gonadotropin secretion (rather than gonadotropic potency of the pituitary) is a limiting factor in stimulation of the ovary in the pre-pubertal animal. Follicular waves have been observed prior to puberty and during other periods of anestrus (Adams, 1999; Ireland *et al.,* 2000), but their complete development and maturation can occur only when circulatory FSH and LH reach adult profiles (Hafez, 1993); and only follicles that secrete adult profiles of estrogen can ovulate in response to the LH surge (Hafez, 1993).

In the study of Sorensen *et al.* (1959) to determine the effects of nutrition on the development of endocrine potencies and activities in the growing pre-pubertal heifers the absolute and relative weights (i.e. weight per hundred pounds of body weight) were measured, and contents of the anterior pituitaries and ovaries were estimated. They reported that, the absolute weight of the anterior pituitary increased while its weight relative to body weight decreased with increasing age and with more intensive feeding (Table 2.3).

Table 2.3: Mean whole pituitary weights and mean pituitary relative weights (grams) of heifers raised on 3 levels of nutrition and slaughtered at 6 different ages. (Relative weights are in brackets).

Level of Nutrition	Age at slaughter (weeks)						Mean (all Ages)
	0-1	16	32	48	64	80	
Low		0.67 (0.42)	1.96 (0.42)	0.95 (0.27)	1.31 (0.28)	1.42 (0.26)	1.26 (0.33)
Medium	0.57 (0.66)	1.16 (0.53)	0.12 (0.32)	1.42 (0.26)	1.44 (0.19)	1.52 (0.17)	1.33 (0.29)
High		1.18 (0.42)	1.46 (0.31)	1.13 (0.18)	1.74 (0.20)	2.03 (0.18)	1.54 (0.26)
Mean (all levels)	0.57 (0.66)	1.00 (0.45)	1.51 (0.35)	1.22 (0.24)	1.49 (0.22)	1.65 (0.20)	

Mean pituitary weights per hundred pounds of body weight given in brackets. Source: Sorensen *et al.* (1959)

This observation suggests that nutrition influences the physical development of the endocrine gland, just like other parts of the body such that low nutrition results in smaller absolute size pituitaries as against those of moderate and high nutrition, at puberty. However, the patterns of the relative weights of the glands indicate reducing rates of development with age and nutrition compared to the rest of the body. This, perhaps, is to ensure that the right adult proportional size of the gland is attained at puberty. In this case low or restricted nutrition may delay puberty physiologically in animals that reach chronological pubertal age but lack functional (secretory) adult size pituitary. In addition, the decreasing relative weight of the pituitary with age and nutrition suggests also that the pituitary's density (mass: volume ratio) is near constant at any time while that of the body varies. The mass of the pituitary will seem to be directly proportional to its volume, and hence the capacity or total content at any time. Practically the mass or weight-size may measure the endocrine potency (hormone content), and hence biosynthetic function of the pituitary.

That the weight-size of the pituitary gland is related to biosynthetic function, that is total hormone content (potency), and rate of secretion, is inferable from Gombe and

Hansel (1973). In a study, they observed that the total content and concentration of progesterone in corpora lutea (CL) were evidently a reflection of the sizes of the CL. In addition, Sorensen *et al*, (1959) demonstrated that thyroid stimulating hormone (TSH) potency and rate of secretion in the anterior pituitary were directly related to the size of the gland, which in turn depended on the nutrition and age of the pre-pubertal heifer. They estimated TSH potency in anterior pituitary (AP) of the heifers by measuring the thyroid acinar cell heights of hypophysectomized rats injected with extracts of the bovine pituitaries prepared under standardized conditions (Table 2.4)

Table 2.4: Mean thyroid acinar cell heights (μ) of rats injected with pituitary extracts from heifers raised on 3 levels of nutrition and slaughtered at 6 different ages.

Level of Nutrition	Age at laughter (weeks)						Mean (all ages)
	0-1	16	32	48	64	80	
Low	8. 50	7.45	7.97	7.94	7.46	7.26	7.61
Medium		8.50	7.80	7.99	7.39	7.39	7.72
High		8.88	8.96	7.89	7.42	7.60	8.15
Mean (all levels)		8.13	8.24	7.94	7.42	7.42	

Source: Sorensen *et al.* (1959)

They reported that the analysis of variance of these data revealed significant differences in TSH potency of the pituitary glands at different ages ($P < 0.05$), there being a gradual decrease in TSH activity (or potency) from birth to 80 weeks of age. This suggests that endocrine potency of pituitary decreases during pre-pubertal growth, from a relatively high level at birth to normal level at puberty. Arthur (1975), states that pituitaries of pre-pubertal animals contain plentiful gonadotropins.

In addition, there was a definite tendency toward higher TSH activity with higher levels of feeding. The TSH content of the pituitary glands from these heifers was related to the actual rate at which the hormone was being secreted. The relative thyroid weights of the heifers were positively correlated with the thyroid acinar cell heights of the rats injected with pituitary extracts from the heifers ($r = 0.60$; $P< 0.01$). A positive correlation was also found to exist between the TSH potencies of the pituitary glands and the rates of growth of the heifers during the 16 – week period before their slaughter ($r = 0.45$; $P < 0.01$).They, therefore, drew the conclusion that:-

- Increased plane of nutrition increased TSH content of the anterior pituitary (AP).

- The TSH content of the AP reflects the rate at which it is being secreted, as indicated by the relatively high correlation between the TSH content of the heifers' pituitary glands and their thyroid weights per unit body weight.
- The thyroid acinar cell heights in heifers increase as the level of feeding increases. This indicates an increased output of the thyroid hormone (Thyroxine) in response to an increased secretion of the pituitary TSH as level of feeding increases. This implies that increased level of nutrition increases the rate of secretion of the two hormones TSH and Thyroxine by the AP and thyroid glands, respectively.

It seems that nutrition may impact gonadotropic function maturation of the pre-pubertal animal by influencing both the gonadotropin potency and secreting activity of the AP. According to Bellows *et al.* (1973), gonadotropins are proteinaceous in nature, and any change in overall metabolism that increases protein synthesis may eventually lead to higher pituitary potency. Moreover, in goats, high energy (glucose) availability is required for increased secretion of the hypothalamic peptide hormone, GnRH (Ohkura *et al.*, 2004).

The other organ of importance in the determination of time of puberty due to nutrition is the ovary. Physiologically, puberty is marked by the first spontaneous ovulation (Lavasseur and Thibault, 1980, cited in Valencia *et al.*, 1990). Sorensen *et al.* (1959) studied the influence of nutrition on ovarian activity of pre-pubertal heifers raised on 3 levels of feeding and slaughtered at 6 different ages and found that both the absolute and relative ovarian weights increased with increasing level of feeding. Marked increases in ovarian weight occurred at about the time of first estrus/ovulation (i.e. onset of puberty between 37 and 50 weeks), in the high– and medium-fed heifers. Thus, the increase in the ovarian weights, both absolute and relative, must have been due to the development of follicles in the ovaries; and this follicular growth is influenced by the rising plane of nutrition. Sorensen *et al*. (1959) further observed as many as 20 atretic follicles per section in the ovaries of the pre-pubertal heifers. These early atretic follicles were characterized by a fibrous-appearing fluid, a degenerated granulosa layer and a connective tissue that trapped the ovum. It is now established that follicular waves do develop in pre-pubertal ruminants (Adams, 1999; Ireland, 2000), and that such early follicles suffer atresia because of unavailable pulsatile LH surge (Lucy, 2003).

According to Wildeus (1996) the weight-size of the ruminant ovary depends on the stage of the ovarian cycle. Sorensen *et al*. (1959) reported further that mature follicles developed earlier, with higher levels of estrogen and progesterone; and the onset of estrus with first ovulation occurred earlier, in the life of heifers on the high level of feeding. These heifers had first estrus at 37 weeks, weighing 596 Ibs (270.3kg), which was earlier, compared with 49.1 weeks, 590 Ibs (270. 8kg) and 70-80weeks, 800Ibs (362.9kg) for the medium and low feeders, respectively. Thus, Sorensen *et al.* (1959) demonstrated that increasing plane of nutrition matures the ovarian activity of the pre-pubertal female animal by:

- increasing the absolute and relative ovarian weights, indicating its potency of follicular development
- delaying atresia of developing ovarian follicles
- causing the rapid maturation of large antral follicles, proximal to first estrus,
- increasing rate of sex steroid secretion, and
- promoting early onset of puberty

Although they did not find any much adverse effects of nutritional level on ovarian activity once estrous cycle had started at puberty, it is now known that severe under nutrition is able to bring about the cessation of all reproductive activities regardless of other factors (Walkden-Brown, 2001).

During physiologic maturation, increasing plane of nutrition promotes early development of the endocrine potency and secretory ability of the hypothalamo-pituitary-ovarian axis; and also enhances the processes of folliculogenesis and steroidogenesis in the ovary. This enables an early adjustment between the increasing gonadotropic and ovarian activities, resulting in production of the first preovulatory follicle(s) and pulsatile LH surge for the first ovulation and estrus, indicative of the onset of puberty and reproductive cyclicity. At and from this stage onwards the doeling can produce progeny if successfully mated in each estrous cycle, with a prolificacy, which normally has an upper limit determined by the ovulation rate (i.e. number of ova shed per estrous cycle). However, the ovulation rate is itself affected by several genetic and environmental factors; the most important of which is the nutritional status of the doeling/doe in each estrous cycle.

2.4.4-3. The Cyclic Phase Effects and Mechanism of Nutrition

In the ruminant female animal once sexual maturity is attained and first ovulation occurs at puberty, the yearling continues to ovulate at a cyclic interval (Arthur, 1975; IAEA, 1984), which in the goat averages 18-21 days (Hafez, 1993). However, severe under nutrition may not only reduce the ovulation rate which sets the upper limit for prolificacy (Hafez, 1993; Walkden-Brown, 2001), but is able to bring about cessation of estrous cyclicity regardless of other factors (Walkden-Brown, 2001). Therefore, the main impact of nutrition on prolificacy of the cyclic female animal is through its influence on the physiology of sustaining the estrous cycle and the ovulation rate in each cycle.

However, both the estrous cycle (i.e. interval between the incidence of two consecutive ovulations) and the ovulation rate (number of ova shed at the end of the interval) are basically controlled by the same hormones – gonadotropins and steroids (Peacock, 1996; Spearow, 1999) from the hypothalamus–pituitary axis and the ovaries, respectively. Ultimately, then, dietary intake acts at various levels within the hypothalamus–pituitary-ovarian axis to control, essentially the ovarian activity – folliculogenesis, oocyte maturation (or quality) and ovulation (Webb *et al.,* 2004). Follicular growth continuum is primarily controlled by gonadotropins and locally produced growth factors; but a number of environmental factors, such as nutrition, can influence both the growth of follicle and quality of its oocyte (Garnsworthy and Webb, 1999). For example, short term changes in the plane of nutrition have been shown to influence small antral follicle (1 to 4mm) recruitment without affecting circulating concentration of FSH (Gutierrez *et al.,* 1997; Armstrong *et al.,* 2001; Gong *et al.,* 2002a) resulting in a larger number of ovulations after a superovulatory gonadotropin challenge (Gong *et al.,* 2002a). Diet has also been positively correlated with the growth rate and size of the ovulatory follicle (Rhodes *et al.,* 1995; Mackey *et al.,* 1999; Bossis *et al.,* 2000). During lactation, especially in dairy cattle, the extent of the negative energy balance deficit is a major factor controlling follicle growth (Butler, 2000). In addition, Boland *et al.* (2001) reported that oocyte competence development is linked with intake of dietary energy. Lucy (2003) noticed that a miniscule energy is required for the neuromuscular mechanisms of the follicle, which lead to extrusion of the oocyte during ovulation. Similarly, egg pick-up, after ovulation, by the fimbriae also requires energy. Hafez (1993) concluded that the oviductal contractions (including those of the fimbriae kinocilia) are believed to be associated with glycogen content of the oviductal musculature.

The detailed physiological mechanisms through which nutrition exerts many of these effects are yet to be fully characterized (Webb *et al*., 2004). However, it is known that extraovarian factors, such as metabolic hormones, and locally produced growth factors are involved in mediating these nutritionally induced changes in follicle dynamics and oocyte quality (Fig.2.7) (Webb *et al*., 2004). Notably among them are growth hormone (GH), Insulin, Insulin-like growth factors (IGFs) and Leptin.

2.4.4-3.1 Growth Hormone (GH)

Growth hormone (GH) also called somatotropin, is a polypeptide hormone, which is involved in general anabolic effects; and stimulates release of insulin-like growth factor-I (IGF-1) (Zubay *et al.,* 1995). It is secreted by the anterior pituitary and stimulates the liver to produce IGFs. Fogwell (1997) observed that concentrations of GH in blood are increased by negative energy balance (NEB) and exogenous GH augments NEB transiently. Further investigations with injected exogenous GH showed increased number of small antral follicles and decreased number of large follicles in cows (Lucy *et al.,* 1992, cited in Fogwell, 1997; Lucy, 2003). Thus the overall effect of GH on the ovary is a reduction on the functional significance of a dominant follicle (Fogwell, 1997). This is supported by Webb *et al*. (2004) as they report that some *in vitro* experiments have shown that GH does not affect the proliferation and steroidogenesis of bovine granulosa cells in serum-free culture. The dominant follicle is supposed to be more steroidogenic in the cohort of follicles (Hafez, 1993). Secondly, GH influences recruitment of small antral follicles, perhaps by the reduction in the dominance effect of the largest follicle. Dominance stops recruitment of follicles in sheep (Hafez, 1993).

The mechanism by which GH affects this inventory of follicles is not clear (Fogwell, 1997), but the suggestion that GH is involved in mediating the actions of nutrition by acting directly on follicles has also been questioned (Webb *et al.,* 2004). This is because mRNA encoding GH receptor is not detected in granulosa cells of bovine follicles (Fogwell, 1997; Webb *et al.,* 2004). However, an *in vivo* somatotropin dose-response study demonstrated that GH acts through increased peripheral concentration of insulin and/or IGF -1 to alter follicle development in heifers (Gong *et al.*, 1997). Furthermore, the recruitment of small antral follicles during acute dietary intake occurred despite decreased circulating GH concentrations, and no differences in peripheral FSH concentrations (Gutierrez *et al*., 1997; Armstrong *et al.,* 2001, both in Webb *et al.,* 2004). This implies that some other factors must also be involved as associates or intermediaries of GH since normally GH concentration in circulation rises with NEB (Fogwell, 1997). Moreover, follicle recruitment usually requires a transient rise in circulating FSH (Webb *et al.,* 2004). It can be suggested that GH acts via other metabolic hormones, such as insulin and IGF-1, to influence follicular development (Webb *et al.,* 2004), especially, recruitment of small antral follicles and decreasing the number of the dominant follicle through predisposition to atresia. For, according to Hafez (1993), interruption of estrogen production at any step results in atresia of follicles.

2.4.4-3.2 Insulin

Insulin is a polypeptide hormone, secreted by the islets of Langerhans of the pancreas. Its primary function is to stimulate uptake of circulating glucose by body cells, lipogenesis and general metabolism (Zubay *et al.,* 1995) and in this way makes both energy and carbon-skeleton available for intracellular biosynthetic functions. The role of insulin in regulating glucose and amino acid uptake by cells of many tissues is well established (Etherton, 1982, in McCann and Hansel, 1986). Thus insulin is believed to be associated with physical or body weight development, although in humans, its effect as predictor of weight gain or physical development is controversial in non-diabetic children and adolescents (Rhodes *et al.,* 2005).

Irrespective of any controversies surrounding its role in body weight gain, insulin (together with leptin) is implicated as one of the circulating indicators of adiposity (Xu *et al.,* 2005) suggesting that insulin and leptin are prototypic metabolic hormones believed to sense and signal the central nervous system (CNS) in the long-term regulation of energy homeostasis (Havel, 2001; Xu *et al.,* 2005). Circulating insulin concentration is proportional to body fat content, and insulin concentrations decrease during fasting and energy-restricted diets, independent of body fat changes, ensuring that feeding is triggered before body energy stores become depleted (Havel, 2001). It is in performing its primary role as a metabolic hormone in both sensing and signaling peripheral energy (nutritional) state in the central regulation of energy homeostasis, that insulin becomes bioavailable to stimulate GnRH release, and thus implicated in affecting reproduction. According to Salvi *et al.* (2006), energy balance exerts a critical influence on reproduction via changes in the circulating levels of hormones such as insulin; and that this modulation of the neuroendocrine-reproductive axis ultimately involves variations in the activity of hypothalamic neurons expressing GnRH, culminating in changes in ovarian activity. Consequently, insulin is positioned to serve as an important link between nutrition and reproduction, since its availability in circulation is greatly dependent on dietary macromolecules.

Butler (2000) associated decreases in dairy cow fertility with NEB postpartum and decreased IGF-1 and insulin concentrations. The initiation of the first ovulation is delayed in dairy cows selected for high genetic merit for milk yield and this has also been shown to be associated with a lower circulating insulin concentration (Webb *et al.*, 2004). In contrast, feeding diets specifically designed to increase circulatory insulin concentrations during early lactation can advance the first ovulation post partum (Gong *et al*., 2002b). Similarly, infusion of insulin into beef heifers increased both the diameter of the dominant follicle (Simpson *et al.*, 1994) and ovulation rate in energy deprived heifers (Harrison and Randel, 1986).

As a metabolic-reproductive mediating hormone insulin may act on either the CNS or the ovaries, directly or indirectly because these are the main biological systems involved in regulating fundamental reproductive functions such as gametogenesis and sexual behavior. That insulin acts on the CNS is well established. According to Brüning *et al.* (2000) insulin receptors (IRs) and insulin signaling proteins are widely distributed throughout the CNS. In a study to find the physiological role of insulin signaling in the brain, they developed mice with neuron-specific IR-genes disrupted. They report that IR signaling in the CNS plays an important role in regulating energy disposal, fuel metabolism and reproduction. In addition Badman and Flier (2005) state that the particular portion of the CNS where signals reflecting energy stores, recent nutritional state, and other parameters are integrated is the hypothalamus; which suggests that the reproductive target cells for insulin in the CNS are in the hypothalamus. Meanwhile, central control of energy balance depends on the ability of two hypothalamic neurons – the proopiomelanocortin (POMC), and agouti-related protein (Agrp), - to sense and respond to changes in the peripheral energy stores, as conveyed or signaled by changes in circulating levels of hormones like insulin (Xu *et al.,* 2005). Moreover, the reproductive response of the hypothalamus due to changes in the circulating levels (and hence, stimulation) of insulin is the expression and secretion of GnRH (Salvi *et al.,* 2006). These data altogether suggest that the reproductive actions of insulin involve actions in the brain and, more specifically, the arcuate nucleus and ventromedial hypothalamus, which, according to Elmquist *et al.,* 1998) are important areas for controlling GnRH release and sexual behavior, respectively.

It appears that the question of whether insulin stimulates the GnRH neurons directly or through neuronal intermediaries is getting cleared. Salvi *et al*. (2006) concluded strongly in a recent study that GnRH neurons are directly sensitive to insulin. In addition, it is evidential that the actions of insulin in mediating the effects of nutrition on reproduction may not be carried alone. Indications are that possible relationships exist with leptin, and perhaps other factors. In a study Jose *et al.* (2005) investigated whether insulin and leptin share common intracellular signal transduction pathways and to determine whether these hormonal signaling systems modulate each other’s actions in rat hypothalamus. They observed a convergence of leptin and insulin at the level of insulin-receptor substrates (IRSs) / phosphatidylinositol 3-kinase (PI3-kinase) and a divergence at the level of Akt, and a direct and positive cross-talk between insulin and leptin at the level of Janus kinase-2 (JAK-2) and signal-transducer-and-activator of transcription-3 tyrosine phosphorylation (STAT-3). This mechanism may increase stimulation in physiological processes such as the control of food intake and body weight, which are under the combined control of insulin and leptin.

In hypothalamus, insulin’s routes of stimulation or signaling circuitry appear to be IRSs – PI3kinase – Akt and mitogen–activated protein–kinase (MAP–kinase) pathways (Jose et al., 2005; Xu *et al.,* 2005; and Salvi *et al.,* 2006). According to Jose *et al.* (2005), whereas insulin activates both IRSs-PI3-kinase-Akt and a MAP-kinase pathway, leptin activates MAP-kinase and IRS-PI3-kinase signaling only, with no effect on Akt, the level at which they diverge. In addition, Xu *et al.,* (2005) showed that leptin and insulin act in parallel to stimulate P13K in POMC neurons but in opposite ways in Agrp neurons. According to Xu *et al.* (2005) POMC neurons are anorexigenic (disfavor food intake, body building and ovulations) while Agrp neurons are orexigenic (favor food intake, energy reservation with improved ovulation). These observations suggest that insulin and leptin may exhibit synergism or positive cross-talk along the P13K pathway in POMC neurons, leading to increased stimulation of anorexigenic physiological processes such as reduction in food intake and body weight (Newswender and Schwartz, 2004) but they may be antagonistic in Agrp neurons - perhaps as a mutual check on each other in regulating their stimulation of orexigenic processes that positively influence GnRH expression, and ovarian activity eventually.

McCann and Hansel (1986) performed an in-depth study to determine the relationships between insulin, glucose metabolism and pituitary-ovarian functions in fasted heifers, in which plasma insulin and glucose concentrations were measured, among others. The fasting treatment lasted between days 8 and 16 of the estrous cycle. Their results showed that:

a) Concentrations of insulin and glucose were decreased ($P<0.05$) by 12 and 36h, respectively, after fasting was begun and did not return to control values until 12h (insulin) and 4 to 7 days (glucose) after fasting ended;
b) LH levels were lower ($P<0.01$) in fasted than in control heifers during the last 24h of fasting.
c) Concentrations of LH increased abruptly ($P<0.01$) in fasted heifers in the first 4h after they were re-fed on day 16 of the fasted cycle.
d) Mean concentrations of LH were greater ($P<0.05$) in fasted (11.2 ±2.6ng/ml) than in control (4.7±1.2ng/ml) heifers during estrus of the cycle after fasting. This elevated LH was preceded by a rebound response in insulin levels in the fasted re-fed heifers, with insulin increasing from 176±35pg/ml to 1302±280pg/ml between re-feeding and estrus of the cycle after fasting.
e) Concentrations of LH, glucose and insulin were similar in both groups after day 2 of the post-fasting cycle.

They concluded that these findings suggest that insulin availability could affect pituitary production of LH or hypothalamic production of GnRH by affecting the level of glucose or amino acid metabolism in these tissues.

Recently, Ohkura *et al*. (2004) demonstrated that glucose availability, but not fatty acids, regulates the pulsatile secretion of GnRH by the hypothalamus. Thus, the reproductive effect of insulin in the hypothalamo-pituitary axis is closely associated with its regular function of enabling glucose and amino acid uptake and utilization by the endocrine cells of these tissues. McCann and Hansel, (1986) found out that both the anterior pituitary (AP) and hypothalamus of ruminants and non-ruminants metabolize substantial amounts of glucose, but the rate of utilization is several-fold greater in hypothalamus than in the AP. Glycolysis and provision of substrate precursor for amino acid synthesis are important fates for glucose in these tissues (Anderson *et al.,* 1961, cited by McCann and Hansel, 1986). The presence of specific binding sites for insulin in the external region of the median eminence in the rat (van Houten *et al.,* 1979) suggest that insulin could impact directly on the metabolism of glucose in this neural tissue and thus modulate release of hypothalamic releasing factors. In addition, insulin enhances GnRH–induced secretion of LH by pituitary preparations *in vitro* (Sen *et al.,* 1979; Adashi *et al.*, 1981). An experiment was conducted to evaluate the effects of dietary restriction on ovarian, endocrine [ovarian steroids and luteinizing hormone (LH) pulse] and metabolic [glucose, insulin and non-esterified fatty acid (NEFA)] profiles in goats treated with a progesterone-releasing controlled internal drug releasing (CIDR-G) device (Tanaka *et al.,* 2004). Results showed that body weight was significantly ($P < 0.05$) decreased in the food-restricted animals. Estrous behavior and ovulation followed by a rise of plasma progesterone concentration were observed after the CIDR-G removal in all control animals but not in any of the food-restricted animals within 12 days after CIDR-G removal. The LH pulse frequency from 32 to 40 h after the CIDR-G removal was significantly ($P < 0.05$) lower in the food-restricted animals than in control animals (1.5± 0.6 versus 3.8±0.5 pulses for 8 h). There was no significant difference in the glucose concentration in weekly plasma samples between control and food-restricted animals. Insulin concentrations from 2 weeks after the start of feed restriction were significantly ($P < 0.05$) lower in restricted animals than in control animals. The NEFA concentration in restricted animals was significantly ($P < 0.05$) increased after the start of feed restriction, and then decreased gradually to the basal level. Their conclusion suggest that nutritionally induced anovulation after CIDR-G treatment is associated with a reduction in the frequency of LH pulses, and that low insulin and high NEFA levels, rather than the glucose concentration in the circulation, may be associated with the metabolic suppression of LH pulses.

McCann and Hansel (1986) observed that re-feeding of fasted heifers 10.00h on day 16 of the fasted cycle caused striking increases in their plasma LH concentrations, indicating that the mechanisms for synthesis and secretion of LH were still intact – in fasted heifers. However, results during re-feeding were in contrast to the decreased plasma concentrations of LH found in fasted heifers before re-feeding. They suggested that secretion and not synthesis of LH (potency) was preferentially affected by fasting; and that this agreed with other data showing that pituitary concentration of LH (i.e. LH potency of pituitary) was unaffected by fasting and underfeeding (Howland, 1972; and Beal *et al.*, 1978). NEB may not affect the gonadotropin sysnthesis and potency of the pituitary as observed by Mtimuni (1995) who expects such to be the case for the gametogenetic phase of reproductive bioenergetics. Furthermore, the possibility of potency not being as limiting as secretion of gonadotropins in the pituitary may be explained from the statement of Zubay *et al.* (1995) that stimulation of peptide hormonal secretion is usually coupled with an increase of hormone synthesis, so that hormonal stores are replenished (always). This further supports the notion that reduced circulating levels of gonadotropins may not often result from reduced pituitary synthetic potency, but rather due to either reduced pituitary secretory activity or increased rate of LH catabolism (degradation), since most hormones have a half-life in blood of only a few minutes (Zubay *et al.*, 1995).

McCann and Hansel (1986) speculated that there might be a physiological link between gut function and control of LH secretion, which was manifested during refeeding of fasted heifers. Hodson *et al.* (1984) suggested that afferent fibres of the vagus nerve and its effect on cholecystokinin may be a part of this physiological link between the gut, hypothalamus and the pituitary. Havel (2001) affirms that changes in circulating dietary macromolecules like glucose, fatty acids and amino acids, as well as gastrointestinal peptide hormones (e.g. Cholesystokinin), form part of the numerous short-term peripheral signals from the gut, working in concert with insulin and leptin to influence the central regulation of energy homeostasis.

Thus the increased blood concentration of dietary macromolecules after refeeding must have signaled and induced an increase in the GnRH and LH secretion-followed-by-synthesis cycle in the fasted-refed heifers. Additionally, that LH levels were greater in fasted than control heifers during estrus and metestrus of the estrous cycle after fasting was explained with the theory that metabolism rate increases in fasted-refed tissues. Tepperman *et al.* (1970) and Field and O'Dea (1980), observed that rebound responses in the intracellular activity of enzymes of intermediary metabolism and increased tissue sensitivity to insulin are characteristic findings in fasted-refed animals; while glucose utilization in anterior pituitary of fasted-refed rats was greater than that in fed controls (Goodner, 1965, in McCann and Hansel, 1986).

McCann and Hansel (1986) noticed further that when LH levels increased in the fasted-refed heifers more than controls, fasted heifers had been refed for 4 to 6 days during which period they had increased concentrations of insulin. These findings, together, led them to conclude that reduced concentrations of LH during fasting, the abrupt increase in LH during estrus and metestrus of the cycle after fasting are all explainable on the basis of changes in the frequency and amounts of GnRH released into the portal circulation and/or changes in pituitary responsiveness to GnRH stimulation – all resulting from changing levels of plasma glucose and insulin reaching the hypothalamus and pituitary. According to Gunn and Doney (1971) nutritional flushing (as a nutrition-reproductive technique) must be viewed physiologically as causing a period of rapid change in metabolism, which may influence onset of estrus and ovulation rate. This must result from increased nutritionally induced biosynthesis and secretion of GnRH and gonadotropins (at the hypothalamo-pituitary axis) that stimulate increased folliculogenesis and steroidogenesis (at the ovarian level). In contrast, animals already well-fed and in good body condition, do not often respond to nutritional flushing (Kinne, 2000); and this may be because the rebound response is absent with such nutritional status, when blood dietary macromolecules may be already in a steady state.

The argument up to this point is that insulin and glucose metabolism does affect reproduction, especially the occurrence of first estrus postpartum and ovulation rate. This happens as a secondary but consequentially coupling effect with the primary functions of insulin. The mechanism is that in performing its primary task of enhancing cellular uptake and utilization of dietary glucose and amino acids (Etherton, 1982) insulin influences a consequential intracellular metabolism of reproductive macromolecules at both the brain and ovarian levels. At the brain level the actions of insulin result in biosynthesis and secretion of GnRH and gonadotropins by the hypothalamic arcuate nucleus and anterior pituitary's (AP) gonadotropes, respectively. This comes about as the metabolism of glucose avails energy (ATP) and carbon skeleton (substrate precursors) (Anderson *et al*, 1961, in McCann and Hansel, 1986) for increased protein synthesis in the respective endocrine cells of the hypothalamus and AP. That this mechanism is more efficient in fasted-refed tissues as a rebound response; and that in such responses it is the secretion rather than biosynthesis of gonadotropins which is increased was demonstrated by McCann and Hansel (1986). This may help to explain why lean, and not fat animals, respond more favorably to flushing.

At the ovarian level a number of studies have demonstrated the importance of insulin as a signal mediating the effects of acute changes in nutrient intake on follicle dynamics (Webb *et al.*, 2004), resulting in an increase in the diameter of the dominant follicle (Simpson *et al.*, 1994), the ovulation rate in energy deprived animals (Harrison and Randel, 1986) and the initiation of first ovulation postpartum (Butler, 2000). In addition, chronic or acute underfeeding affects function of the corpus luteum by a carry-over inhibitory effect (of nutrition) on the preovulatory follicle of the preceding cycle (McCann and Hansel, 1986).The action of insulin on the ovary seems to be mediated by a feedback mechanism between it and estrogen; that is, the availability of one is in turn influenced by the availability of the other. Webb *et al*. (2004) noticed that increases in serum insulin levels parallel the increase in estrogen associated with the development of the dominant follicle. They cited Morimoto *et al.* (2001) who showed that estrogen stimulated both the expression of mRNA encoding insulin and its secretion from the pancreas in a number of species. Moreover, they themselves correlated diet-induced increases in circulating concentrations of insulin with increased estradiol production in cultured granulosa cells from small antral (1 to 4mm) follicles. This latter observation is a demonstration of a direct action of insulin on follicle function (Webb *et al.,* 2004). A large number of granulosa and theca cell *in vitro* studies have demonstrated the direct action of metabolic factors (Webb *et al.,*2004) (Fig 2.6). Indeed, cell culture studies have shown bovine granulosa cells to be critically dependent on the presence of physiological concentrations of insulin (Gutierrez *et al.,* 1997b).

These observations support the belief that insulin has a direct action on follicle growth and function by direct stimulation of steroidogenesis in granulosa cells of both small and preovulatory antral follicles, thereby reducing rate of atresia and enhancing chance for more follicles to be selected and mature for ovulation. Recently, Butler *et al.* (2004) showed that insulin infusion increased estradiol secretion by the dominant follicle of the first postpartum follicular wave in dairy cows, and this effect appears not to be mediated through changes in pulsatile LH release. Hafez (1993) stated that a high level of estrogen production, which stimulates growth and cell differentiation of granulosa, is central to selection of a given follicle for maturation and ovulation; and hence avoids atresia of follicles. This then may explain the effects of dietary-induced increase in availability of insulin during the estrous cycle; as well as the significantly increased concentrations of insulin during the preovulatory period observed by McCann and Hansel (1986) and Armstrong *et al*. (2001).

2.4.4-3.3 Insulin-like growth factors (IGFs)

As with insulin, there is increasing evidence linking nutritionally induced changes in systemic IGF-1 concentrations with ovarian activity (Webb *et al.,* 2004). The liver is the main source of systemic IGF-1 and GH is the primary regulator of hepatic IGF-1 gene expression and secretion (Zubay *et al.*, 1995). However, insulin treatment also results in increased circulating total and free IGF-1 concentrations in dairy cows (Butler *et al.,* 2004). In addition, estrogen has been shown to be linked to changes in insulin, increase concentrations of GH, increase in IGF-1 secretion and circulating IGF-1 concentrations in ovariectomized cattle (Webb *et al.,* 2004). Diet induced changes in circulating levels of the components of the IGF-1 system have also been described (Clemmons and Underwood, 1991; Thissen *et al.*, 1994; Monget and Martin, 1997) with circulating IGF-1 concentrations being positively correlated with level of feeding (Vandehaar *et al.,* 1995; Rausch *et al.*, 2002).

It appears that nutritionally induced increases in circulating levels of insulin and estrogen may act with GH to increase hepatic IGF-1 secretion into circulation (Webb *et al.,* 2004). It has been demonstrated that IGF-1 as well as insulin, interact with FSH to stimulate granulosa cell estradiol production (Spicer *et al.*, 2002), since both IGF-1 and IGF-1 mRNA have been detected on granulosa cell cultures, of both small and large follicles.

Apart from hepatic IGF-1, other growth factors from local ovarian sources are also involved in follicular dynamics. In the cow, IGF-II gene expression is restricted to the theca of small antral follicles, providing support that IGF-II is the major intraovarian IGF; and there is general agreement that IGF-II acts through IGF-1 receptor (Webb *et al*., 2004) during recruitment of small antral follicles upon stimulation of FSH (Armstrong *et al.*, 2001).

Insulin-like growth-factor-binding proteins (IGFBP) also have a regulatory role in follicle development, by controlling the bioavailability of IGFs (Thisssen *et al.*, 1994). Peripheral concentrations of binding proteins are regulated by dietary intake (Rausch *et al*., 2002). However, these effects may be modulated by other metabolic factors such as insulin, which has been shown to decrease peripheral IGFBP-2 concentrations, but not affect IGFBP-3 concentrations (McGuire *et al.*, 1995). Control of IGF by IGFBP is important because excess IGF may have a negative effect on oocyte growth (McCaffery *et al.*, 2000). Local intraovarian IGFBP-2 seems to help regulate follicle selection during follicular development. In healthy bovine antral follicles up to 9mm in diameter, IGFBP-2 and -4 mRNA expressions are restricted to granulosa and theca tissue, respectively (Armstrong *et al.*, 1998). Moreover, Austin *et al.*, (2001) observed that IGFBP-2 and possibly IGFBP-4 concentrations are higher in the follicular fluid of small- and medium-sized follicles and that of large atretic follicles, but are undetectable in the follicular fluid of large and/ or dominant follicles. The implications are that the absence of IGF-binding proteins in the larger (9mm size) follicles makes both systemic and local IGFs more available to stimulate granulosa estradiol synthesis and secretion, while it is vice versa in the subordinate follicles. This then will favor steroidogenic dominance effect and selection of the larger follicle(s). The conversion of a subordinate follicle to a future dominant follicle has been associated with a transient increase in follicular estradiol, but a decrease in IGFBP-2 (Kojima *et al*., 2003).

It may, therefore, be that the increased insulin during follicular phase of estrous cycle (McCann and Hansel, 1986) inhibits expression of IGFBP-2 in the future dominant follicles in a cohort, and thereby increase the chance for more than usual number of follicles to eventually respond to the ovulatory LH pulsatility, resulting in increased ovulation rate. Generally, the IGF system is involved in follicular cell differentiation and steroidogenesis by enhancing the action of gonadotropins (Souza *et al.*, 2002) especially that of FSH during follicle recruitment, culminating in possible high ovulation rate. It is hypothesized that increased dietary energy decreases the steady-state concentration of mRNA encoding IGFBP-2 and -4 in medium-sized antral follicles, which in turn increase bioavailability of locally produced IGF-II and systemically derived IGF-I in these cells, (Webb *et al.*, 2004) during follicle recruitment.

Lucy (2003) believes that nutritional mechanisms that increase gonadotropin pulsatility through their actions on the hypothalamus and pituitary also co-ordinate simultaneously, an increase in the responsiveness of the ovary to the gonadotropins (Fig.2.1).

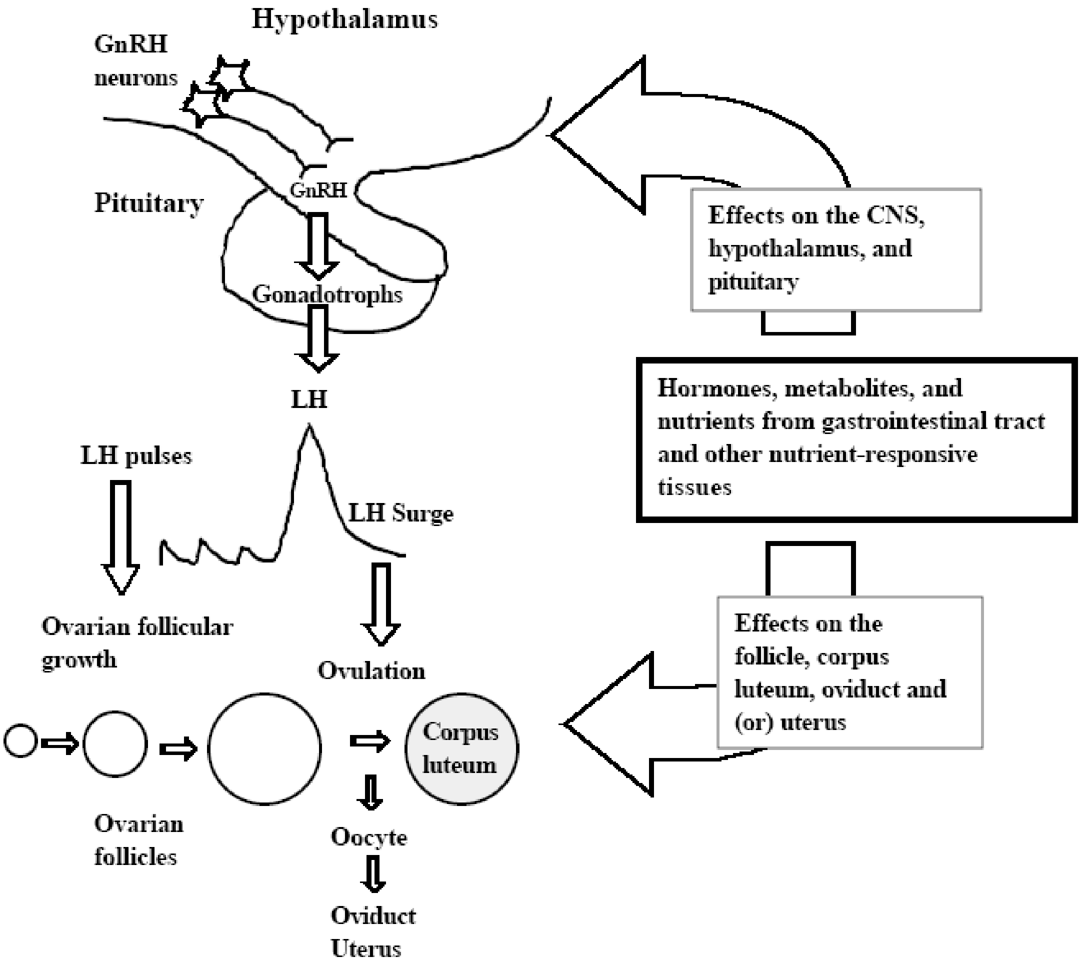


Figure 2.1 Conceptual model for the mechanisms through which nutrition affects reproduction in postpartum cows

Hormones, metabolites, and nutrients from the gastrointestinal tract and nutrient-responsive tissues affect GnRH and LH secretion through their actions on the central nervous system (CNS) and hypothalamus. These same hormones and metabolites may have direct effects on ovarian function (both follicles and corpora lutea) as well as the oocyte, oviduct, and uterus. The combined effects of each axis determine postpartum fertility Source: Lucy, 2003.

2.4.5 The Effects of Management

According to Devendra and McLeroy (1992) management or husbandry practices can significantly modulate the effects of genetic and some environmental factors affecting reproduction rate in domestic animals. The application of such practices is based on understanding of the physiologic mechanisms by which they affect reproduction (Wildeus, 1996) and the economic appropriateness for the enterprise (Walkden-Brown, 2001). Wildeus (1996) explained that the utilization of reproductive management techniques has only a limited application in an extensively managed herd, but can be a useful tool to improve performance of a more closely managed herd. This is because in applying biotechniques such as for estrus synchronization, multiple ovulations, artificial insemination and embryo transfer in large farms, additional/special inputs will be needed in labour (including skill), handling facilities and in the area of nutritional provisions. In SSA, even though flock sizes are relatively more closely manageable, general economic recession may render application of commercial exohormones less cost effective. In the U.S., most of the commercial exohormones developed for reproductive manipulation in goat and sheep are not available and/or approved for use, and their use, if any, has been mainly on experimental basis (Wildeus, 1996). In addition, Walkden-Brown (2001) noticed that superovulation in goats is readily achieved with gonadotropic hormone preparations such as pregnant mare's serum gonadotropin (PMSG) and FSH, but this is generally restricted to treatment of embryo transfer donors. Moreover, whereas there is a role for use of exohormones in lower doses for the induction of out of season breeding, workers in France have revealed that goats develop antibodies to repeat doses of PMSG leading to declining efficacy of ovulation induction with repeat treatments (Walkden-Brown, 2001).

However, natural reproductive manipulations without (or with limited) aid of exohormones may be the choice where the enterprise cannot sustain use of strictly artificial bio-techniques (Walkden-Brown, 2001). Such natural methods include sociosexual (or male effect), nutritional (or flushing) and controlled lighting (or photoperiod) treatments (Wildeus, 1996). Probably, the most useful natural means for increasing ovulation rate (and hence prolificacy) in does is by flushing (Walkden-Brown, 2001). While hard data is not available for goats most goat breeds will show greater ovulatory responses to improved nutrition effect (or flushing) than will Merino sheep which show an increase of 1.2 to 2.0% in ovulation rate for each kg additional body weight at the time of mating (Walkden-Brown, 2001). It is suggestive, therefore, that the most economically effective and relatively quicker natural biotechnique to use managerially for reproductive manipulation of goats in an economic-stressed SSA may be programmed nutritional flushing.

2.4.5-1 Flushing as a Nutritional Biotechnique

According to Findlay and Vaughan (1964), the concept of flushing arose when it was observed and accepted that ovulation rate in sheep could be influenced by nutrition. It became an agricultural practice widely adopted since the start of the 20th century (Robinson, 1973), but its definitions vary and are mostly unclear (Gunn and Doney, 1971).

For example, nutritional flushing has been described as “increased plane of nutrition before mating” (Spedding, 1973) or “feeding in such a way that the ewes put on little weight at the time of mating” (Banerjee, 1976). None of these descriptions precisely give any economic time frame for flushing before mating, type of feed and state of the animal or any direct expected reproductive effects. Earlier, however, Thomson and Aitken (1957) gave a clearer insight of the definition of nutritional flushing when, upon a review of the results of many workers, they defined nutritional flushing as the practice which consists of giving ewes that are in fairly poor condition an improved diet for a few weeks before mating so that they are in rapidly gaining condition when they meet the ram. They emphasized that giving of extra feed to ewes which are already in good condition does not constitute flushing, and this emphasis was later given evidential support by Gunn *et al*. (1969) who found in an experiment that the level of feeding before and at mating had a positive effect on both ovulation rate and lambing rates of lean but not moderately fat ewes. Gunn and Doney (1971) explained that a period of rapid change in metabolism and/or body condition (due to flushing) can stimulate both onset of estrus and the number of follicles that produce ova. Miller and West (1972) were more specific on the timing and effects of flushing and gave a dictionary definition of flushing as: the process of inducing a condition of rising metabolism in breeding ewes some 6 to 3 weeks before tupping, by putting them on to protein-rich food stuff; the practical effect of this is to ensure that each ewe is in fit but not fat condition to breed, and intensify heat or estrus.

Nix (2004) explains that nutritional flushing in simple terms refers to putting the animals on a higher plane of nutrition 30 days prior to and 30days after breeding, before being returned to maintenance diet. The technique normally involves using a supplement high in energy and/or protein, with the purpose of facilitating better ovulation rates and increasing implantation, conception and twinning rates. Since the goal of flushing is to cause the does to gain weight and body condition thin does in poor condition will tend to respond somehow favorably to, but does that are in excessive body condition will likely have no response or may actually respond negatively to flushing.

Walkden–Brown (2001) also states that nutritional flushing is probably the most useful natural means of increasing ovulation rate in does because it ensures that does are in good body condition (static effect) and are increasing in live weight (dynamic effect) leading up to and during mating. According to Cox *et al. (*1987) in the long term, nutritional level determines both body weight and condition while in the short term it is related to an increased level of nutrients at intracellular level, stimulating the release of gonadotropic hormones or acting directly in the ovary by increasing its metabolism and function.

These definitions point out significant technical considerations that have informed modern understanding and practice of nutritional flushing. They show, in the first place, that flushing must be limited to poor-conditioned animals, which must be provided with protein- and energy-rich feed for, at least a total of 3-weeks before and/or through mating. Secondly, the effects of flushing include induction of rising metabolism (dynamic effect) and increased ovulation rate with improved body condition/weight (the static effects). From physiological point of view, flushing may be defined as a nutritional intervention by which a high plane of nutrition induces a rapid increase in the metabolism of poor body conditioned does, (within a total period of about 3-8 weeks) during the mating season, resulting in increased ovulation rate in the short term, and improved body condition/weight in the long term, with possible advancement of estrual onset. Practically, therefore, the effects of flushing are both dynamic and static in terms of time, and in terms of measurable parameters, involves changes in the physical status (or body condition/weight) and physiological functions (especially levels of gonadotropic and ovarian activity) of the doe before and/or during mating.

2.4.5-1.1 The Discrete (Static) Effects of Nutritional Flushing

In the long run flushing results in changing the body mass or physical status of the doe (Cox *et al.* (1987, cited by Meza-Herrera *et al.,* 2004). This means that the doe's body condition score or live weight moves from one level to the other over time, and assumes a new physical status at mating. This new physical status then is known as the static physical effect of flushing (Walkden-Brown, 2001). Coop (1962) stated that live weight has been established as a major component of the effects of nutritional flushing; and Spedding (1970) expressed a view that about half the total effect of nutritional flushing appears to be due to liveweight and that where this is already adequate the response to flushing may be quite small. This view is still held in the field as expressed by Nix (2004) who states that does in excessive body condition will likely have no response or may actually respond negatively to nutritional flushing.

In an experiment to determine the effect of six week supplementation on body condition of ewes, Gunn *et al*. (1969) reported having obtained a substantial gain in condition score. The level of gain, quality and level of their ration were not, however, stated. Blasu (1981) did not obtain any significant gain in the body condition of Djallonke ewes fed on basal *Cyanodon plectostachyus* (14%CP) and *Stylosanthes guyanensis* (15.10%CP) forages, having crude fibre contents of 33.1% and 32.2% respectively. The ewes were supplemented with locally made concentrate (TDN = 69.65%, CP =11%) for varying periods between one and three weeks, in Ghana. Using the hexo-point scale, the score range of 1.0 to 3.0 and the experimental flock modal score of 1.5 remained before and after flushing. Since the study was done during the rains when lush grazer was in abundance and could itself alone improve the body condition of the animals (Mowbray, 1974), Blasu (1981) incriminated insufficient duration of flushing, and /or a possibility that the score range 1.0 to 3.0 may be natural and optimal for this breed of sheep in their native environment, in which case within a year even with longer period of supplementation it will only be expected to move the percentage of ewes in lower scores towards 3.0. According to Oliver and Broadbent (1972) cited by Oppong-Anane, (1975), within a flock, the range of conditions at any one time will unlikely be more than two scores, (i.e. full score intervals) though the optimal range may change with time and season.

Hart *et al*. (1999) studied the effect of flushing on ovulation and conception rates and litter size in 96 multiparous Spanish does using energy and protein concentrates. Corpora lutea, pregnancy status and litter sizes were determined by ultrasound. They reported that the gains in BCS of does flushed prebreeding with protein cum energy supplement (SP) (57%) was significantly more (P<0.10) than in the controls (C) (39%), as well as those given longer term energy (LE): 40d 0.25kg/d/doe corn-based supplement (30%) and short term energy (SE): 20d 0.25kg/d/doe corn-based supplement (38%). It appears from their results that the energy cum protein supplementation may be more effective than energy supplement alone in improving the body condition of does; and that dietary protein /energy (P/E) ratio intake may have to be well balanced for efficient muscle and fat build up in goats.

The importance of balanced dietary P/E ratio lies in the fact that muscle is basically a deposition of protein; and, according to Kinne (2004) the changes in BCS of (Pigmy) goats between scores 1 and 3 (on the pento-scale) is more of muscle build up. Moreover, the conversion of excess energy through fatty acids and triacylglycerols for deposition by and in adipocytes is regulated by enzymes whose synthesis itself depends on the rate of transcribing DNA into RNA (Zubay *et al.,* 1995). Since both DNA and RNA synthesis require nucleobases from amino acids, dietary protein may limit the overall process by limiting availability of starting amino-acid-substrates for the syntheses of fatty-acid-metabolase-mRNA and the fatty-acid-metabolases.

Hart *et al*. (1999) did not report on the effect of nutritional flushing on body weight of the does in their study. However, Coop (1969) estimated that, in practice, flushing can result in 6-8% gain in body weight. Blasu (1981) observed that, though some of the ewes in his study lost weight over the 3-week period, about 60% of the ewes in each treatment group (including the controls) gained some weight. The group that consumed a cumulative total of 5.8 kg / head of supplement in the 3 weeks recorded the highest percentage (87%) of ewes gaining weight, but their average percent weight gain (11.49%) was not significantly better ($P > 0.05$) than the corresponding values for ewes not supplemented or supplemented for shorter durations. It was explained that probably the basal forage grazer provided equally good nutrients for all the groups, and that the period of supplementation was perhaps too short for appreciable difference in weight gain to be established. Similar results were obtained by Barton and Asiedu (1972) in the same country, Ghana. They reported that non-supplemented WAD goats made weight gains which were comparable to those for goats grazing and receiving supplement as well. The crude protein levels of the forage they used were between 3.4% and 6.6%, with crude fiber contents between 26.1% and 31.3%. The fortified silage and wheat bran in their supplement had 9.2% CP and 17.4% CP, respectively. A report similar to theirs, also for WAD young goats, was given by Asamoah-Amoah (1975). These two reports did not indicate the duration of supplementation nor assigned reasons for their results.

Apart from the static effect on physical status, nutritional flushing induces also static physiological parameters of ovarian activity of the female animal, in the long run. According to Spedding (1970), the ultimate or static response of the ewe to flushing is the increase in her lambing performance or litter size at birth (prolificacy). However, prolificacy depends largely on ovulation rate at mating. According to Gunn and Doney (1971), within normal physiological limits there is a genetic maximum number of ova shed in any one estrous cycle; and this varies between breeds (Hafez, 1993; Kinne, 2000). Where the maximum is very low (1.0 – 1.2) variation due to nutritional effects is likely to be slight, unless low nutrition results in and increases the number of females failing to ovulate (McKenzie and Terrill, 1937). Conversely, where the maximum is very high (3.0 – 4.0) it is probable that nutritionally created variation could be high, though some breeds might have ovulation largely independent of nutrition (Gunn and Doney, 1971). Generally, however, it has long been accepted that ovulation rate can be influenced by nutrition (Findlay and Vaughan, 1964) and around this has arisen the concept of nutritional flushing. Therefore, through its influence on the ovulation rate at mating nutritional flushing can affect the prolificacy at birth in the long run.

The static effectiveness of nutritional flushing on reproduction has technically been demonstrated in terms of increased ovulation rate (Allen and Lamming, 1961). However, it has often also been expressed simply as larger crops of progeny or prolificacy (Vita, 1951) due to the rather expensive nature of experiments involving ovulation counts (Edey, 1969). Gunn and Doney (1971) concluded that the long-standing apparent discrepancies and inconclusiveness of the ultimate effect of nutritional flushing on reproductive performance of female animals may be largely blamed on the fact that many observations and experiments suffer the disadvantage of using parturition percentage as the index rather than ovulation rate. Since the inception and practice of nutritional flushing to date reports on its static reproductive effect have been controversial (Miller and West, 1972; Hart *et al*., 1999). While some earlier workers showed clearly in ewes that flushing increased ovulation rate (McKenzie and Terril, 1937), twinning rate (Vita, 1951), and lambing percentage (Hulet *et al.*, 1962), others, like Briggs *et al.*, (1942) were unable to substantiate the beneficial effects on lambing rate. Vita (1951) emphasized that the increase in multiple birth due to nutritional flushing is to be found chiefly in those ewes which for genetical reasons, tend to produce twins or triplets.

Similarly, Larsen *et al*. (1975) and Blasu (1981) did not find any significant improvement in lambing performance due to flushing in Djallonke ewes. The latter worker reported lambing and twinning percentages of 108% and 5.4%, respectively and observed that these performances were lower than the corresponding rates of 112 – 130% (Baah, 1980) and 9.6 – 50% (Jollans, 1960; Oppong-Anane, 1971) reported by previous investigators. Brown (1972), working on Nungua Blackhead ewes concluded that flushing could yield some benefits in this breed of sheep in Ghana. In the study of Hart *et al. (*1999) corpora lutea, pregnancy status and litter size were measured by ultrasound. They reported that flushing did not increase ($P > 0.05$) fetal number (1.59, 1.74, 1.88, 1.78) and ovulation rate (2.45, 2.17, 2.21, 2.29) for the 40-d energy only (LE), 20-d energy only (SE), 20-d energy + protein (SP) and non-supplemented (C) groups, respectively. They concluded that nutritional flushing did not improve ovulation rate, conception rate or litter size of Spanish does. This might be because the animals were probably already in reasonably good body condition (2.3) before flushing started. Hart (2005, personal communication) commenting on the subject holds the view that whereas there may be potential to increase kidding rate in very thin or poorly conditioned animals with nutritional flushing the real solution is a better all round nutritional management. He stressed that if the does are given special nutritional attention by flushing only when they are being bred, in case they have nutritional stress later they may absorb the fetus, have a small kid or not raise a good kid.

Meza-Herrera *et al* (2004) evaluated the effect of by-pass protein supplementation level (PSL) and body condition (BC) upon ovarian activity and serum insulin (INS) concentrations in 32 Saanen-Alpine X Criollo goats in Mexico. In a 2 x 2 factorial of complete randomized block design, the goats (n = 32, 19 mo), with BC either low (LBC, n = 16, BW = 28.81± 0.72kg, BC = 2.1± 0.3), or high (HBC, n = 16, BW = 35.12±0.72kg, BC = 3.2± 0.3) received one of two PSL's: without protein (NPROT, Og/goat/d) or protein (PROT, 120g/goat/day) during a forty-day preovulation period. Estrus was synchronized and during the late luteal phase (post-ovulation) total ovarian activity (TOA) was evaluated by transrectal ultrasonographic scanning, considering the total number of follicles (FT) and corpora lutea (CLT). They reported that protein supplementation affected CLT and FT ($P<0.05$) favoring the PROT–goats (2.62 ± 0.21 v 2.06 ± 0.21, and 2.68 ± 0.25 v 1.92 ± 0.17, respectively). They concluded that dynamic effect (by – pass protein supplementation) increased ovarian activity in these goats.

One can deduce from the two studies above that:

1. The use of ultrasonographic technology can help determine the actual technically expected effects of nutritional flushing on reproduction – ovulation rate and/or fetal numbers – and thereby make experimental results more comparable with reduction in discrepancies that stem from differential indices (Gunn and Doney, 1971).
2. Although both studies measured the ultimate effect of flushing on reproduction in terms of ovarian activity results still show discrepancy. This may be due to genetic or breed differences (Gunn and Doney, 1971; Hafez, 1993), the body condition of the does before flushing and the different nutrients emphasized in the flushing. Apgar *et al.*, (1975) expressed a view that nutritional treatments need not have a single effect on the reproductive system. With at least three sites that can be affected, which according to Hafez (1952) are the brain, ovary and accessory reproductive tissues, and with several nutrients that could become limiting, it is probably not surprising that results obtained with nutritional treatment (whether by overfeeding or underfeeding) have been conflicting.
3. It appears that flushing’s benefits (improved body condition and increased ovarian activity) may be better reaped in goats by using rations with a well balanced protein/energy ratio. Hart *et al.* (1999) influenced higher change in body condition with an energy – protein than energy alone supplement, and Meza-Herrera *et al.*, (2004) showed that greater ovarian activity was favored by protein supplementation. These observations may throw challenge to and may require a review of the old notion, at least in goats, that protein is less important than energy in the reproduction of farm animals (Mukasa-Mugerwa, 1989). For goats, this review of notion is recommendable because goats

deposit less fat in body than sheep (Peacock, 1996) agreeing with Kinne (2004) that changes in body condition scores from 1-3 (on pento-scale) of goats is more of muscle than fat. Moreover, goats are unable to utilize nutrients as effectively as sheep, though they select feed of higher nutritive value if given the chance (ILCA report, 1987). According to Nix (2004) supplements high in energy and protein are best for increasing body condition and thus reproductive performance in thin and moderate condition does.

2.4.5-1.2 Relationship between the Static Physical and Physiological Effects of Flushing

Results of investigations and scientific opinions have been published with respect to the relationship between the physical status and reproductive parameters of female animals. Tribe and Seebeck (1962) indicated that the liveweights of ewes at tupping, and to a lesser extent before flushing, are related to the subsequent lambing performance. Coop (1962) reported that twinning rate of ewes that were heavier prior to flushing was greater than that of lighter ewes to the extent of 6% per 10 1b (4.5kg) increase in weight. Hulet *et al*. (1962) found that ovulation rate following flushing was significantly correlated with initial and final weights of the ewe.

These early workers found a relationship between body weight and reproductive activity in sheep, but the role or contribution of flushing to this relationship was not clear since both initial and final weights following flushing were implicated in one way or the other. Around the same time arose an idea that body fat-mass could also relate reproductive performance, and with it came the development of a system for scoring body condition in Australia (Mukasa-Mugerwa, 1989), originated by Jefferies (1961). According to Hileman *et al*. (2000), when it was observed that the increase in biomass predicts reproductive activity it was hypothesized that attainment of a critical body weight (Kennedy and Mitra, 1963) and or fat mass (Frisch, 1980) – among others – must be achieved to induce first estrus, and, possibly, ovulation rate at puberty and postpartum anestrus. Oppong-Anane (1975) demonstrated in British sheep with graphical analysis that body condition score was more precise than liveweight in accounting for high incidence of lambing. This report, which lent credence to that of Gunn *et al.* (1969) showed that for British breeds of sheep the optimum score appeared to be 3.0 (on the hexo-scale) above which the level of pre-mating food intake had no effect on ovulation rate, but below which food intake was important. He concluded that body condition *per se* may be indicative of subsequent lambing performance. Working with Djallonke ewes in Ghana, and using the same score-scale and graphical analytic method, Blasu (1981) reported that, apparently, both body condition score and bodyweight at mating due to flushing tended to accurately indicate the subsequent lamb production, in agreement with Cumming (1977), though body condition score was slightly better. He found that ewes with condition scores 1.0, 2.5 and 3.0 at mating had 100% lambing rate, while those in 1.5 and 2.0 had 105.26% and 112.5% respectively. The optimal condition score, therefore, appeared to be 2.0. With regard to liveweight of the ewe at mating, ewes in the extreme weight ranges 15-19kg and 30-34kg had 100% lambing. The corresponding rates for ewes in the mid-weight ranges 20-24kg and 25-29kg were 107.69% and 108.33%, respectively. He felt that two optimal class weights may exist in the flock (22kg and 27kg for the small and big-sized animals, respectively) at which lambing rate could improve. A similar observation was made by Oppong-Anane (1975) who noted two clearly defined optimal – 60kg and 100kg – in six British breeds. Pooling results over the whole experimental flock Blasu (1981) concluded from the graphical presentation that body condition at mating may be a more precise determinant of subsequent lamb production than liveweight at mating in our local ewes.

The need to determine optimal body weight for reproduction is not only for physiological purposes, but also has economic undertones in the goat production enterprise. According to Žujović *et al.* (2004) considering the fact that goats with increased body weight should realize higher production, body weight can be used as one of the selection parameters. However, increased body weight has the consequence of increased food consumption, among other measures, justifying the need for determining optimal economic body weight of goats for production. In a recent study to determine optimal body weight of Domestic White goats in hilly-mountainous region of Stara Planina in Belgrade, Serbia, Žujović *et al.* (2004) found correlation between body mass of doe at mating and various production traits. They reported having established that increased number of twin kids was positively related to body weight of goat dam at mating but gave no correlation/regression coefficient. In addition there was a weak ($P>0.05$) and negative correlation between doe body weight at mating and duration of pregnancy, which pointed to the tendency of shortening pregnancy with increase of dam body weight such that the shortening was more expressed in does bearing twins (BW = 50kg, r = -0.1766) than in singleton (BW = 46kg, r = -0.1243). Contrarily, positive correlation was observed between birth weight of kids and body weight of does at mating, and birth type of kids. Generally the birth weight of female kids, whether of single (r = 0.4196, P< 0.01) or twin (r = 0.0730, P > 0.05) birth demonstrated stronger correlation with dam body weight at mating than for male singles (r = 0.0213, P> 0.05) and twins (r = 0.0703, P> 0.05). After considering these relationships and those of the other measured traits – duration of lactation, body mass of kids at weaning, and daily gain in weight of kids up to weaning – Žujović *et al.* (2004) concluded that optimal body mass of Domestic White does in, order to obtain optimal kid production (including prolificacy and weaning rate), was 46kg for singletons and 40kg for twinners. They explained that lighter goats had somewhat more tendency for higher ovulation rate and milkability and, hence, higher weaning rate. Impliedly, it is both physiologically and economically prudent to manage nutritional flushing such that pre-breeding body weight of our local does is fit but not fat.

2.4.5-1.3 The Dynamic Effects Of Flushing

The dynamic effects of flushing on the female animal are described variously as liveweight changes (Tribe and Seebeck, 1962), or rapid increase in food supply and hence in body condition (McKenzie and Edey, 1974) or rapidly rising body condition (Coop, 1962) or a gaining plane of nutrition to stimulate higher ovulation rate (Wildeus, 1996) or increasing energy intake (LUARE, 2000), or increasing in liveweight (Walkden-Brown, 2001). All these descriptions connote the sense of rapidity and hence shortness of time within which both physical and physiological parameters (resulting from metabolism, endocrine and ovarian function) tend to change. The belief was that flushing did not influence reproductive activity by only its long term static but also short term dynamic effects. However, the dynamic effect was explained mainly with physical body changes only. Coop (1962) reported that twinning rate of ewes that were heavier prior to flushing was greater than that for lighter ewes, and added that there was flushing effect resulting from dynamic liveweight; and that considering this dynamic liveweight the time relationship between flushing and mating may be less important. In practice, the emphasis, according to McKenzie and Edey (1974), had changed from implicating only static effects, and to consider flushing as involving a rapid increase in body condition a few weeks prior to mating and so had also frequently involved a deliberate reduction in condition before flushing.

However, the rapid physical body changes *per se* may not make significant practical impact on reproduction in a short term. Cox *et al.* (1987) considered that nutritional level (or flushing) has effects which, in the short term, are related to an increase of nutrients at the intracellular level, stimulating the release of gonadotropic hormones or acting directly in the ovary by increasing its metabolism and function. The implication is that a period of rapid change in metabolism and, hence, body condition, can stimulate both the onset of estrus and the number of follicles which produce ova (Gunn and Doney, 1971). That this concept of positive relationship between rapid metabolism and increased ovarian function exists is inferable from McCann and Hansel (1986). The explanations generally given suggest that metabolism rate increases in tissues that experienced NEB prior to increasing plane of nutrition due to the increased level of circulating insulin under such conditions, especially the influence of insulin in the utilization of glucose and amino acids in the hypothalamo–pituitary – ovarian tissues.

2.4.5-1.4 Relationship between the Dynamic Effects of Flushing and Reproductive Rate

According to Arias *et al.* (1993) and Spicer *et al.* (1993) nutritionally induced changes in serum insulin stimulate GnRH release, provoke FSH secretion and LH pulsatility, increase steriodogenic ability of ovarian follicles and luteal progesterone secretion, as well as maintain normoglycemia. Comparatively lower body scores (leanness) depict tendency to NEB, which according to Whitaker *et al.* (1993) is characterized by hypoinsulenemia, but which is also a reason for rebound responses of nutritionally induced increasing serum levels of insulin and general metabolism, upon flushing (McCann and Hansel, 1986), resulting in increased gonadotropic and ovarian functions (Cox *et al.*, 1987) and increased probability of high ovulation rate and prolificacy (Gunn and Doney, 1971). This suggests that while animals with lower BCS (which is tendency to NEB) exhibit rebound response (natural aggressive reactions to reverse/compensate for loss of metabolic status) in the intracellular activity of enzymes of intermediary metabolism and increased tissue sensitivity to the effects of insulin upon refeeding (Tepperman *et al.,* 1970; Field and O'Dea, 1980) animals that are already in very good body condition (tendency to positive energy balance, PEB) do not seem to exhibit the rebound responses. It may be deduced that the dynamic effects of flushing result from the predisposition of the animal to:

- rebound metabolism by low body condition status,
- increased serum concentration of insulin upon feed intake, and
- increased tissue sensitivity to insulin activity that increases intermediary micrometabolites at cellular levels.

One can thus conclude that the dynamic effects of flushing may be assessed with the relationship between serum level of insulin and the resultant influences on body condition, gonadotropin concentrations and direct ovarian activity (steroid hormone levels, ovulation rate) or to some extent, prolificacy – as indirect measure of ovarian activity.

In the study of Meza-Herrera *et al.* (2004) where they measured serum insulin levels, they reported that goats with BCS of 3.2 had higher ($P< 0.05$) serum insulin level (1.092 ± 0.17ng mL^{-1}) than those with BCS 2.1 (0.81 ± 0.17 ng mL^{-1}), suggesting a positive correlation between serum insulin level and body condition at mating. Similarly they found a positive correlation between level of protein supplementation and serum insulin concentration since does receiving 120g/day/doe protein had 1.69ng mL^{-1} insulin against 1.04ng mL^{-1} for the non-supplemented does. In addition, they found a positive correlation between serum insulin levels and corpora lutea totals (ovulation rate), with $r = 0.46$; $P = 0.06$) and total follicle count, $r = 0.38$; $P = 0.10$). They concluded that insulin levels in serum of goats were affected by both BCS and protein supplementation.

This finding appears to corroborate the statement of Peters and Mayer (1993) that protein supplementation acts as a potent stimulator of insulin secretion. Similarly Muturi *et al*. (2002) evaluated the effect of low and high levels of by–pass protein on plasma glucose, IGF and insulin. While no differences were observed on plasma IGF -1 levels, glucose concentrations differed ($P < 0.05$) at the 60, 90 and 120 min post feeding, and high protein supplementation affected ($P < 0.05$) the plasma insulin concentrations. It can be concluded that, not only will nutritional flushing of does with protein–rich supplement increase serum concentration of insulin, but will also result in positive correlation between serum insulin levels and body condition and ovulation rate. Moreover, insulin may therefore, be affirmed as a metabolic hormone critical in the dynamic effects of nutritional flushing, and that since dynamic effects occur in the short term (Coop 1962; Cox *et al.,* 1987) the critical role of insulin should be demonstrable. Indeed, according to Webb *et al.*, (2004), feeding diets specifically designed to increase circulating insulin concentration during early lactation (time of NEB) has been reported by Gong *et al*. (2002b), who concluded that such feeding levels can advance the first postpartum ovulation / estrus in animals. Moreover, in nutritionally restricted females who received exogonous insulin Almeida *et al*. (2001) reported increase in E_2 and LH pulsatility, diminished follicular atresia, and increased number of pre-ovulatory follicles in the cohort, resulting in high ovulation rate. According to Meza-Herrera *et al*. (2004) the serum insulin concentration of does in NEB during early lactation was found to be around 0.5ng mL^{-1}, while during middle lactation animals in a PEB had insulin level close to 2.5ng mL^{-1}. In addition, infusion of insulin into beef heifers increased both the diameter of the dominant follicle (Simpson *et al.*, 1994) and ovulation rate in energy deprived heifers (Harrison and Randel, 1986). One is then of the view that since the dynamic effects of flushing occur in the short term (Cox *et al.*, 1987) the duration of flushing and the rapidity or dynamic changes in physical parameters may not necessarily be as critical (Coop, 1962) as rapid physiological changes – especially the changing level of circulating metabolites and metabolic hormones (Cox *et al.,* 1987) in affecting reproductive performance. Considering the static and dynamic effects of flushing together it appears that both the eventual gain of body mass and short term increasing level of nutrients, can promote a metabolic status characterised by high serum insulin levels and increased ovarian activity, making insulin an essential metabolic hormone, in the physiologic link between nutrition and reproduction.

2.4.5-2 The Physiologic Basis of Nutritional Flushing

Despite the abundant evidence that nutrition and other environmental factors can exert influence on various aspects of reproductive activity of the female animal, all observations persistently pose the problem of HOW environment exerts its effect and by what mechanism the physical and psychological components of an animal's environment modify its activity (Holmes, 1968). Hafez (1952) suggested that nutritional factors may affect the reproductive process of the female by exerting an influence on the anterior pituitary, the nervous system, sex hormones metabolism, and responsiveness of accessory reproductive tissues to sex hormones. Apgar *et al.* (1975) stated that there are at least three sites on the reproductive axis that can be affected by malnutrition. Yeates (1965) cited the experiments of Allen and Lamming (1961) in an attempt to elucidate the mechanism by which nutrition affects ovulation rate. From these works he concluded that nutrition exercises its effect by regulating the release of gondotropin from the anterior pituitary, and not by direct action on the ovaries themselves. The view was that the main aspect of reproductive physiology likely to be stimulated by nutritional flushing is the neuro-endocrinology of follicle maturation and ovulation (Short and Austin, 1972). Perhaps, because the negative effects of nutrition associated with under feeding were explained with the theory of pseudohypo-physectomy attention was focused on the pituitary and hypothalamus as the primary sites affected by underfeeding (Apgar *et al.*, 1975).

Gombe and Hansel (1973) conducted an experiment in which progesterone and LH were measured in underfed cows. The concentration of progesterone (P_4) in the plasma decreased and the concentration of LH increased. In spite of the increased LH concentration in the underfed cows the size of the corpus luteum was smaller than that in the fed controls. They concluded that the ovary rather than the hypothalamo-pituitary axis had been affected by the reduced food intake. Apgar *et al.* (1975), considering that the observations of Gombe and Hansel (1973) should be demonstrable *in vitro*, conducted a similar experiment, using the same nutritional levels and treatments, and added incubation of the corpus luteum with three levels of LH. They observed that progesterone concentration was only slightly affected, and LH concentration was significantly decreased in the underfed cows, suggesting an *invivo* impairment of the hypothalamic-pituitary axial function. However, during the *in vitro* incubation, LH stimulated the production of progesterone by the corpora lutea from both groups; the increase was less, though, in the feed-restricted group, particularly at the highest level of LH. They explained that since corpora lutea from both groups produced some progesterone, their corresponding ovaries were sensitive to LH stimulation. However, the reduced response to LH by the corpora lutea from cows receiving a restricted feed intake could have resulted from a lack of substrate reserves with which to respond to LH rather than to a reduced sensitivity to LH. Apparently then, *in vivo*, the same restricted feed intake (NEB) that reduced plasma LH concentration must have simultaneously reduced ovarian response to LH stimulation. In this case, contrary to the old belief, nutritional influence on reproduction may have to be seen as impacting at both the brain and ovarian levels. In a review Lucy (2003) expressed belief that hypothetically, nutritional mechanisms that increase gonadotropin pulsalitity through their actions on the hypothalamus and pituitary also coordinate, simultaneously, an increase in the responsiveness of the ovary to the gonadotropins (Fig 2.1).

Sensing and signaling of nutritional status in a biological system may involve direct innervation, availability of circulating dietary macromolecules and/or metabolic hormonal stimulations as the possible mediators. According to Holmes (1968) although there is very little support for a direct innervation of the secretory cells (gonadotropes) of the pituitary there is some evidence that their vessels of blood supply may be innervated, and that by means of variations in the flow of blood (due to the innervation) some influence could be exercised over the gland. McCann and Hansel (1986) suggested, from an experiment that, afferent fibers of the vagus nerve could be a physiologic link between the gut, hypothalamus and the pituitary. Hodson *et al*. (1984) showed that abdominal vagotomy prevented cholesystokinin (a gastrointestinal peptide hormone) inhibition of prolactin secretion by the pituitary in rats while Quigley *et al*. (1981) reported that feeding acutely increased serum prolactin level in humans.

Havel (2001), states that there are mechano- and chemo-receptors for sensing and signaling the presence and energy density of food in the gastrointestinal tract. He further states that changes in circulating dietary macromolecules like glucose, fatty acids and amino acids as well as metabolic hormones like cholecystokinin and insulin form part of the numerous peripheral signals for short term or current level of nutrition from the gut and associated visceral allies, which influence the central regulation of energy homeostasis. In addition, Ahima (2005), in a review showed that there was some evidence in mice suggesting that the transduction of leptin signals may be electrophysiological as observed in the vagal afferents in the gut, arcuate nucleus, supraoptic nucleus and glucose-sentitive neurons in the brain, the last of which is modulated through an ATP-sensitive K+ channel. It appears some amount of current nutritional level may be sensed by gastrointestinal tract mechano- and chemo-receptors and signaled to the brain electrophysiologically, but not without the involvement of metabolic hormones, especially cholecystokinin, insulin and leptin.

There is an overwhelming published data suggesting insulin (Webb *et al.*, 2004; Xu *et al.*, 2005 and Salvi *et al.*, 2006) and leptin (Web *et al.*, 2004; Cassy *el al.,* 2004; Ahima, 2004 and 2005) as the most prominent among metabolic hormones that interact with short term metabolic signalers (Havel, 2001) to produce long term or sustained changes in energy balance and reserves of adiposity. Insulin and leptin are the prototypic metabolic hormones believed to sense either fed or fasted nutritional status and signal the tissues of the hypothalamus, pituitary and ovary, accordingly. Generally, the nutrition–reproduction functions of insulin and leptin are similar in many respects, since, according to Fraley (2004) and Xu *et al.* (2005) both are hormones that directly relay information regarding an individual’s peripheral nutritional status to the brain; where their receptors and signaling proteins are widely distributed (Brüning *et al*., 2000; Ahima, 2005).

The arcuate nucleus of the hypothalamus is important for regulation of energy and GnRH secretion (Elmquist *et al.,* 1998) and is where insulin and leptin modulate reproductive endocrinology (Salvi *et al*., 2006) their receptors co-localize neuropeptides involved in energy homeostasis, especially agouti-related protein (Agrp) and neuropeptide–Y (NPY). These stimulate feeding and induce orexigenic effects. Contrarily, proopiomelanocortin (POMC) neuropeptides – a precursor of other hormones like melanin stimulating hormone (MSH), thyrotropic releasing hormone (TRH) and corticotropic releasing hormone (CRH) - indirectly inhibit food intake, stimulate thermogenesis and induce anorexigenic effects (Xu *et al*., 2005; Ahima, 2005). Insulin and leptin transduce their signals via ligand–receptor–enzyme activation pathways (or circuitries), through either stimulation or inhibition of these neuropeptides (Fig. 2.2). It appears from the review of Ahima (2005), and the study reports of Jose *et al.* (2005), Xu *et al.* (2005) and Salvi *et al*. (2006) that, with a few variations, both insulin and leptin transmit their signals via the following circuitries; depending on the receptor;

(a) JAK – STAT: Janus family kinase–signal transducers and activator of transcription pathway
(b) IRSs – P13K – Akt: Insulin receptor substrate–phosphatidylinositol - 3kinase protein kinase – β pathway
(c) MAPK – Erk: Mitogen–activated protein kinase–extra cellular regulated kinase pathway.

It seems that the concentrations of insulin and/or leptin in the cerebrospinal fluid (CSF) are related to the intensity and/or type of signal. In humans, concentration of leptin is 100-1000-fold higher in plasma than in CSF and correlate positively with fat mass (Schwartz, *et al.*, 1996 cited by Ahima, 2005). The CSF-to-plasma leptin ratio is lower in obesity, suggesting a reduction in efficiency of leptin uptake (in the CNS) (Schawartz *et al.*, 1996). Conversely, the CSF-to-plasma leptin ratio is raised in patients with anorexia nervosa, and might underline the abnormal perception of hunger and body image, and failure of weight gain, in these patients (Mantzoros *et al.*, 1997, in Ahima, 2005).

The CSF concentration of insulin and leptin is regulated by the blood-brain barrier (BBB) (Ahima, 2005), because of their large structural size. In fact the mechanism by which polypeptides that act in the CNS are transported into the brain remains unclear (Ahima, 2005). However, Banks *et al.* (1996) cited in Ahima (2005) showed that leptin entered the rodent brain through a specific saturable mechanism distinct from that of insulin. Moreover, Maness (2000) reported that the BBB transporter of leptin was also not encoded Lep_r as was thought and obesity *per se* is not a crucial determinant of leptin across the BBB. All the same, brain leptin transport in normal animals is partially saturated over a wide physiological range, from low levels associated with fasting to high levels in obesity (Ahima, 2005). There is a close correlation between brain transport of leptin and insulin and their circulating levels such that brain transport of leptin is eliminated (Kastin and Pan, 2000), and that of insulin drastically reduced (McCann and Hansel, 1986) during fasting in parallel with their rapid fall in circulation. Conversely, refeeding increases brain transport of both hormones and this is concomitant with their rising levels in plasma (Kastin and Pan, 2000; McCann and Hansel, 1986). It is inferable from the studies of Kastin and Pan (2000) and Fraley (2004) that these adaptations might involve lipids and/or other nutritional factors, and allow leptin and insulin to function as signals of both satiety and starvation

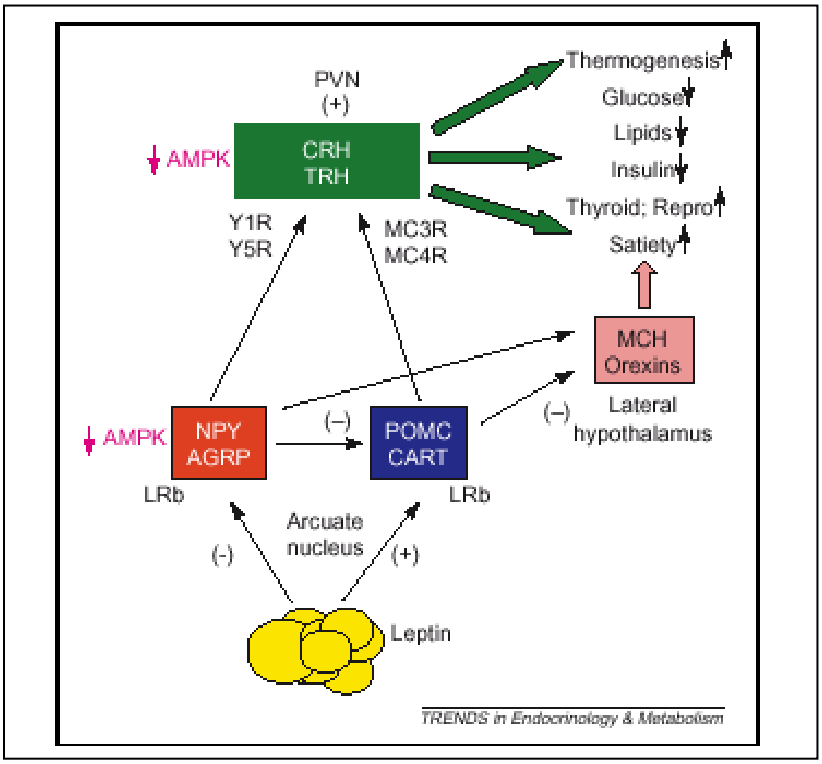


Fig.2.2 Hypothalamic neuronal circuit for leptin

Leptin directly suppresses NPY and AGRP and stimulates POMC and CART neurons in the arcuate nucleus, leading to inhibition of feeding, increased thermogenesis and reduced glucose and lipids. NPY controls feeding via Y1 and Y5 receptors (Y1R and Y5R, respectively). AGRP antagonizes the anorectic action of a-MSH action at MC4 receptors (MC4R). Another effect of leptin is to inhibit AMPK, resulting in stimulation of fatty acid oxidation and weight loss. Source: Ahima (2005)

Apparently, the level and rate of the response depend on the efficiency of the signal transduction pathway (Xu *et al*., 2005) and the physiological (biosynthetic) needs of the body to ensure a steady state distribution of intermediary metabolitites and energy according to the urgency of their needs (Zubay *et al*., 1995). Thus, an increase in leptin, signifying fed or satiety state indirectly inhibits the effects of Agrp and NP-Y, while stimulating increased level of POMC, which eventually mediate inhibiton of food intake, stimulation of glycolysis, thermogenesis and lipolysis (Zubay *et al*., 1995; Ahima, 2005, Fig 2.2).

These neuropeptide-inhibitory actions of leptin tend to produce anorexigenic effects of reduced food intake, NEB and body mass loss (Ahima, 2005). The absence of leptin in the hypothalamus signifies starvation, as may be implied from the works of

Kastin and Pan (2000). From the work of McCann and Hansel (1986) the level of insulin during starvation (and which signifies NEB) must be very low, but not completely eliminated as in the case of leptin (Kastin and Pan, 2000). However, it seems the low-level insulin continues its regular function of stimulating cellular uptake of glucose and amino acids (Etherton, 1982) to sustain vital processes during basal metabolism in all body cells, including hormone biosynthesis and secretion.

The basal insulin stimulates Agrp and NP-Y, resulting in increased food intake concomitant with absence of leptin. Insulin level then increases gradually and signifies increasing food intake, alongside increase of its role in glucose uptake, lipogenesis and general anabolism. Thus the stimulation of neuropeptides by insulin tends to produce orexigenic effects.

The central control of food intake and body weight (or energy balance and adiposity) is associated ultimately with a tendency for negative complementary endocrine effects of insulin and leptin. Xu *et al*. (2005) report a finding that leptin and insulin act in opposite ways on Agrp neurons, and conclude that this may be a new view of hypothalamic circuitry in which the effects of leptin and insulin are not integrated by orexigenic neurons. Jose *et al*. (2005) report a positive cross-talk (additive action) between insulin and leptin at the level of JAK2-STAT-3-tyrosine phosphorylation pathway, which occurring in POMC neurons, suggest anorexigenic synergism. Jose *et al*. (2005) conclude that this mechanism may serve to increase stimulation in physiological process such as control of food intake and body weight, which are under the combined control of insulin and leptin. They also reported a non-additive (permissive cross-talk) at the IRS-P13K level but a divergence at the *Akt* level.

It appears that leptin does not compromise on its anorexegenic effects; it therefore either antagonizes or permits insulin’s orexigenic-stimulations and synergizes with insulin for anorexigenic effects, in their combined control of food intake and body conditioning, through signaling energy regulating neurons in the arcuate nucleus of the hypothalamus. That is to say while insulin and leptin complement negatively in energy homeostasis, they can integrate their actions sometimes, but only to intensify anorexigenic effects, perhaps when there is threat of adiposity to other biological functions, especially reproduction. Most likely the link between reproduction and nutrition comes about with the nutritionally induced bioavailability of leptin and insulin in the arcuate nucleus of the hypothalamus, primarily for regulation of energy balance, but ending in coupling with regulation of the biosynthesis and secretion of reproductive hormones, especially GnRH, whose secretory neurons also occur in the earcuate nucleus. According to Salvi *et al.* (2006) energy balance exerts a critical influence on reproduction via changes in the circulating levels of hormones such as insulin; and that this modulation of the neuroendocrine – reproductive axis ultimately involves variations in the activity of hypothalamic neurons expressing GnRH, culminating in changes in ovarian activity. In an experiment to find relationship between energy metabolism and pituitary-ovarian functions in fasted heifers, McCann and Hansel (1986) found that reduced concentrations of LH during fasting, abrupt increase in LH with refeeding, and greater levels of LH during the estrus and metestrus of the cycle after fasting were all based on changes in the frequency and amount of GnRH released into the portal circulation, and/or in the pituitary responsiveness to GnRH stimulation; and that all these were due to changes in the circulating level of insulin during fasting and refeeding.

Similarly, Ahima (2004) stated that leptin regulates the synthesis and secretion of GnRH, gonadotropins, and sex steroids, such that the decrease in leptin levels during fasting mediates the suppression of reproductive, thyroid, and growth hormones and the elevation in glucocorticoid levels in addition to stimulating food intake and limiting energy expenditure. He cited the work of Welt *et al.* (2004) as further evidence of a link among energy stores, leptin and reproduction from eight patients with hypothalamic amenorrhea (that is caused by underweitght or strenuous exercise), treated with low baseline levels of recombinant human leptin. This resulted in increased mean and pulsatile LH levels, concomitant with enlargement of the ovaries, increased size of dominant follicles, raised extradiol concentration and 65% normalization of menstrual cycle – though with only 38% ovulatory cycles. At the same time, the leptin treatment increased thyroid and growth hormones without adverse effects, and body fat was substantially reduced though food intake and energy expenditure were not affected. The latter observations may suggest a possible permissive interaction between leptin and insulin at the low level of leptin administered. Similar pharmacological treatments with insulin are reported. Infusion of insulin into beef heifers increased both the diameter of the dominant follicle and

ovulation rate in energy deprived heifers (Simpson *et al.*, 1994; Harrison and Randel, 1986).
Moreover, Fraley (2004) reported having shown that only the combination of insulin and leptin administration in male rats with pharmacologically induced diabetes, can fully restore all physiologic measures, including food intake, reproductive physiology, and galanin-like peptide (GALP) expression. Thus pharmacological treatments have also provided good evidence of the involvement of insulin and leptin in simultaneous regulation of both energy and reproduction.

It can, therefore, be concluded that metabolic hormones and factors, prototyped (typically represented) by insulin and leptin, have innate ability for stimulating reproductive effects alongside their primary role in nutritionally induced regulation of energy. Hence, flushing being a nutritional treatment, induces changes in the bioavailability of insulin and leptin, thereby providing opportune chance for them to couple energy balance regulation with reproductive endocrine stimulations; and in so doing complement each other either permissively or antagonistically, perhaps together with other factors, such that the effects of the couple–action are well coordinated between both the brain and ovary (Fig. 2.1)

The exact coupling and complementing pathways are not clearly defined. However, the major conditions and physiologic understandings in this literature review may be summed as follows;

1. Greater positive benefits (static and dynamic effects) of nutritional flushing are obtained in only poor body condition female animals (that is in a state of NEB) (Nix, 2004).
2. Fasted or poor body condition and NEB status seem to prime reproductive tissues to unusually increase metabolism rate rapidly (McCann and Hansel, 1986).
3. The rebound rapid increase in metabolism leads to body mass build up as a static effect in the long term, and increased biosynthesis and secretion of reproductive hormones and ovulation rate, in the short term (Cox *et al.*, 1987, in Meza-Herrera, 2004).
4. The rapid metabolism rate is enhanced by increasing level of circulating dietary metabolites especially glucose and amino acids (Zubey *et al.*, 1995)

and mediated by induced bioavailability of metabolic factors, especially insulin and leptin (McCann and Hansel, 1986; Ahima, 2005).

5. The fasted or NEB (or low body condition) status is characterized also by low insulin and leptin levels in plasma and CSF (McCann and Hansel, 1986; Ahima, 2005), with brain transport (or concentration) of leptin completely eliminated in severe fasting (Kastin and Pan, 2000). CSF levels of leptin correlate positively with plasma levels (Ahima, 2005).
6. A low CSF/plasma leptin ratio favors limited energy expenditure, reduced thyroid activity (and hence low respiration and heart rates, according to McCann and Hansel, 1986), and elevation of glucocorticoids (which may imply a potential for increasing protein synthesis, according to Zubay *et al.,* 1995). It favors also increase in food intake from the stimulation of Agrp and NP-Y neurons in arcuate nucleus of the hypothalamus by insulin (Xu *et al*., 2005; Ahima, 2005).
7. Rising levels of dietary molecules due to flushing induce the bioavailability of insulin and leptin to rise accordingly in both plasma and CSF (McCann and Hansel, 1986; Hileman *et al*., 2000; Ahima, 2005).
8. At the brain level, the rising bioavailability of insulin at moderate concentrations induces orexigenic effects of food intake, cellular glucose and amino acid uptake, increased anabolism (including biosynthesis of GnRH and FSH/LH), and some lipogenesis in adipocytes to reserve energy. Insulin treatment leads to reduced circulating non-esterified fatty acids (NEFA) Butler (2004).
9. At the same time the increasing level of leptin tends to modulate the rate of the insulin-induced adiposity with anorexigenic influences by inhibiting Agrp and NP-Y neuronal effects of food intake, while stimulating POMC neuron to cascade secretion of anorectic hormones, including thyroid hormones (Ahima, 2005).
10. Increased thyroid activity may stimulate increased cellular respiration and heart rates (Sorensen *et al*., 1959), perhaps aimed at reducing adiposity by exhausting glucose and fatty acids, which are the main energy metabolites for heart muscle (Zubay *et al*., 1995), with possible reduction in life span and reproductive performance in life (Sorensen *et al.,* l959).

11. Overall, the anorexigenic actions of leptin result in modulation of food intake, stimulation of thermogenesis, and lipolysis, and hence, regulated rates of body mass build up, and stimulation of reproductive endocrine function (Ahima, 2005), especially secretion of GnRH from GnRH-neurons in hypothalamus and gonadotropins from the gonadotropes in anterior pituitary (Salvi *et al.*, 2006).
12. The low and high changes in concentrations of insulin and leptin in the normal hypothalamus serve as sensation of starvation and satiety, respectively (Hileman *et al.*; Fraley, 2004), and the stimulatory or inhibitory actions of insulin and leptin serve as signals for appropriate orexigenic and anorexigenic responses of the CNS. The signals may be transduced by either ligand-recptor-enzyme activation circuitry or electrophysiologically (Ahima, 2005).
13. When there is excessive food intake with corresponding excess adiposity that threatens other biological functions like reproduction both insulin and leptin may integrate their signals at the LRb-JAK2-STAT3 level in POMC neurons to intensify anorexigenic effects (Jose *et al.,* 2005; Ahima, 2005) – reduced food intake and loss of body condition.
14. With low and rising to moderate body condition levels, however, the prevailing situation seems to be as outlined supra to step (12), resulting in increased secretion of GnRH and subsequent increase in gonadotropins (McCann and Hansel, 1986).
15. The rate of secretions (as against synthesis) of GnRH and gonadotropins is more influenced in the short-term (dynamic effect) by flushing (Sorensen *et al.,* 1959), but the rate of secretion is, however, directly related to the glandular potency (Sorensen *et al.,* 1959).
16. The increased level of gonadotropins (FSH/LH ratio) increases ovarian function that is probably primed simultaneously by insulin and leptin when they are acting on the brain (Lucy, 2003). The gonadotropins act directly on ovarian follicle dynamics in concert with insulin; but the involvement of leptin is either not known (at least in humans, Ahima, 2004) or that leptin inhibits the synergistic interaction between gonadotropins and insulin in ruminants (Spicer, 2001, in Webb *et al.,* 2004), though it may act directly on the ovary to regulate chicken reproductive function (Cassy *et al.,* 2004).

17. As at the brain level, so at the ovarian level, increase concentrations of circulating dietary nutrients due to flushing induce increasing levels of insulin and hence gonadotropins and, therefore, serve as sensor of and signal between nutritional status and follicle dynamics (Webb *et al.*, 2004).
18. Through its stimulation of glucose uptake and increased anabolism (Zubay *et al.*, 1995) and increasing bioavailability of IGF-1 through inhibition of IGFBP – II (McGuire *et al.*, 1995; McCaffery *et al*., 2000; Kojima *et al*., 2003 and Webb *et al*., 2004) insulin influences direct increase in steroidogenesis in granulosa cells – thereby reducing rates of atresia and enhancing chances for more follicles in a cohort to be selected and matured for ovulation.
19. The nutritionally induced effects of insulin in enhancing higher ovulation rate may be in concert with (Webb *et al*., 2004) or without (Butler *et al*., 2004) pulsatile LH.
20. Although there are a host of metabolic factors/hormones with which insulin and leptin may act (or not) in concert, the two appear to be prototypic (Ahima, 2005) even so because their pharmacological therapies have shown results of improving some reproductive problems related to NEB in the case of leptin in humans (Welt *et al*., 2004, in Ahima, 2004) and insulin in cattle (Harrison and Randel, 1986; Simpson *et al*., 1994).

Finally based on the understandings from this review and the report of Fraley (2004) it may be suggested that a critical leptin/insulin ratio will be the more physiologically sensitive long-sought factor linking nutrition and reproduction.

CHAPTER THREE

MATERIALS AND METHODS

3.1.0 Study Location and Area

3.1.1 Study Location

Apart from climatological data, which were obtained from the Ghana Meteorological Service in Sunyani, and also serum chemistry and hormonal analyses at the Kintampo Health Research Centre (KHRC), all other records, observations, and experimental animals were from the National Goat Breeding Station at Kintampo (Fig 3.1.)

Fig 3.1 The research site (National Goat Breeding Station, Kintampo)

The Animal Production Directorate (APD) of the Ministry of Food and Agriculture (MoFA) set up the Station - the only one of its kind in Ghana - in 1996. Its principal objective is to be responsible for the genetic improvement of West African Dwarf Goat in Ghana by:-

(1) Selecting for improved health, growth and reproductive traits, and

(2) Supplying genetically improved bucks (billies) to prospective out-farmers.

In addition, the Station collaborates with Non-governmental Organizations (NGOs) and Research Institutions in programmes that lead to increasing goat production as well as training for their staff. It also undertakes production and supply of forage planting materials to farmers.

The Station covers a total area of one square mile (2.59km^2). At the time of the study about 30.6ha (75.61ac) had been developed. However, most activities had scaled down due to lack of resources, including deteriorating farm structures and equipment. The total stock (does, bucks, and kids) was 544, out of which 40 does were selected for the experiment (Fig.3.2).

Fig. 3.2 A section of the Experimental Animals being introduced to their new room and feeding system

3.1.2 The Study Area

The MoFA National Goat Breeding Station is at Kintampo, which is located on latitude 08° 03´N, longitude 01° 43´W and with an altitude of 373m above sea level. The area lies in the transitional zone between the high forests to the South and interior savannah to the North. The zone is thus sometimes described as derived savannah, characterized by a mixture of tall trees, shrubs, and grasses. The climate is hot and humid (see Table 3.1 and Fig.3.3 for meteorological data, 2000-2004).

Monthly temperatures range from 14.8°C to 36.6°C, with an annual average of 27.0°C. The highest maximum and minimum temperatures occur in March, with averages of 35.6°C and 23.5°C respectively. Relative humidity varies from 90% in the morning (0900hrs local time = GMT) during the wet season to as low as 21% (at 1500hrs) in the dry season. The rainfall measurements for the five years (2000 - 2004) ending just before the study started, averaged 1300.6mm per annum with 69.2% occurring between April and October (inclusive), and the rest 30.8% from November to March.

Table 3.1 Climatological data of Kintampo from 2000-2004 (Source Ghana Meteorological Service, Sunyani)

Month	Rainfall (mm)	Temperature ^{0}C		Relative Humidity (%)	
		Maximum	Minimum	09:00Hrs GMT	15:00 Hrs GMT
January	24.4 (0.0 – 70.8)	34.2 (33.3–34.0)	20.2 (14.8-21.9)	51.4 (36-71)	31.6 (23-46)
February	30.2 (0.0– 107.7)	35.4 (34.5–36.5)	22.5 (20.8–23.5)	52.0 (37– 74)	28.4 (21-39)
March	36.2 (7.3 – 65.1)	35.6 (34.5–36.6)	23.5 (22.7–24.1)	68.2 (64-72)	37.8 (23-47)
April	163.1 (114.6-229.7)	33.4 (32.6-32.6)	23.0 (22.5-23.2)	75.8 (73-77)	59.8 (53-64)
May	133.1 (56.9-212.6)	31.9 (31.1-32.6)	23.0 (22.6-23.1)	80.4 (80-81)	64.4 (61-67)
June	199.4 (142.9-255.9)	30.4 (29.9-32.9)	21.8 (21.7-21.9)	84.0 (82-88)	71.0 (69-71)
July	132.3 (31.3-291.7)	28.7 (28.3-29.4)	21.5 (21.3-21.8)	86.4 (84-88)	73.8 (69-78)
August	147.1 (53.2-280.9)	28.3 (27.7-28.5)	21.4 (20.8-21.6)	86.0 (82-89)	73.4 (71-79)
September	198.5 (141.4-253.2)	29.1 (28.7-29.5)	21.5 (21.4-21.9	86.0 (84.90)	74.0 (71-77)
October	185.0 (93.0-274.8)	31.4 (30.2-32.5)	21.8 (21.2-21.9)	79.8 (76-82)	68.2 (61-71)
November	43.2 (31.1-92.4)	32.2 (31.4-32.7)	22.5 (21.6-22.8)	79.8 (78-82)	59.6 (52-63)
December	8.1 (0.0-24.4)	32.8 (32.0-34.1)	20.9 (17.3-22.5)	55.4 (58-81)	39.0 (17-56)
	Total=1300.6mm p.a	Monthly mean=32.0	Monthly mean=22.0	Monthly mean= 73.8	Monthly mean=56.7

NB: Figures in brackets are ranges

Although a slight dip in precipitation tended to occur in May and July/August, the entire seven (7) months of 69.2% rainfall was considered the wet or rainy season for the purpose of this study since animal feed did not seem to be lacking during this period. The other period of five (5) months, was then the dry season when precipitation was not only 30.8% but temperatures were highest – ranging from

20.2°C to 36.6°C – while animals and most pastoral vegetation (grass, shrubs and small trees) suffered severe dehydration and wilting, respectively.

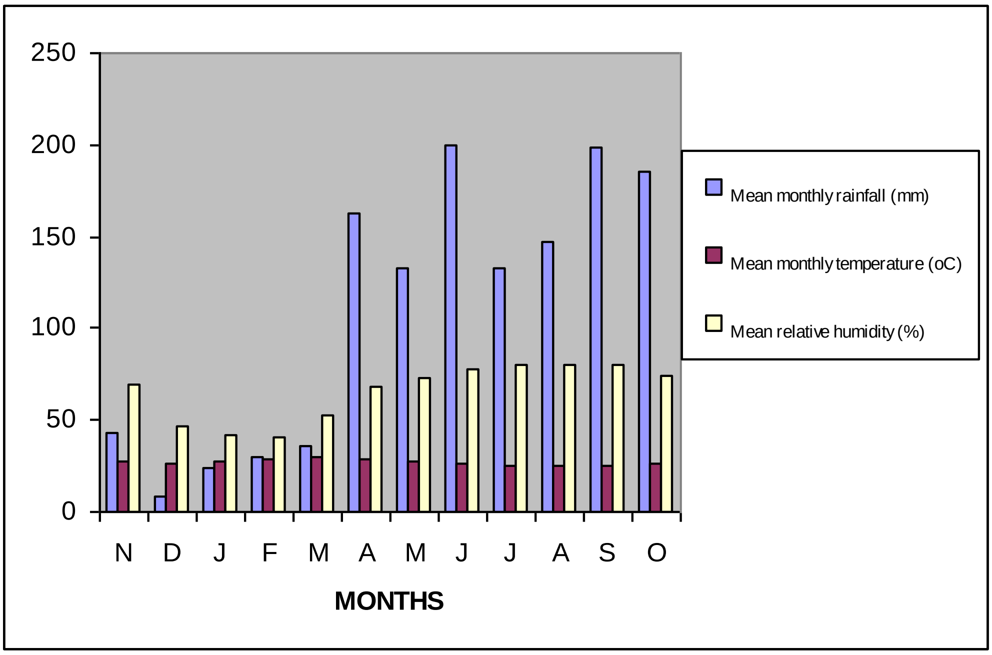


Fig.3.3 Column Chart of Climatological data of Kintampo (2000 – 2004)

3.2.0 Experimental Design and Set-up

The study, which covered the period between April and October, 2005 was in three parts:

(1) Flushing with feed supplement, to improve the body condition of the selected experimental does, prior to mating.

(2) Assay of serum chemistry (glucose, total protein, calcium) and hormones (insulin and gonadotropins), to determine the quantitative nutritional status and reproductive hormonal levels respectively of the does at mating; and

(3) Monitoring of parturition to note the types of birth and litter sizes at birth.

3.2.1 The Experimental Design

The animals used in this study were West African Dwarf (WAD) breed of goats being kept at the Station for genetic improvement (breeding) purposes. They were made up of forty does aged between 3 and 6 years, which had kidded at least three times. In addition, six bucks known to be of good fertility (though not proven in this work) were used for mating the does.

The flushing experiment was a two-by-two (2^2) factorial setting involving two levels each of SUPPLEMENTATION and AGE. Twelve weeks before the start of the study the 40 lactating does were kept away from bucks to ensure no service on return to post-partum cycling. On the starting day (April 18, 2005) the kids were weaned at 3 to 3.5 months old and the does divided into two groups of 20 according to age: 3-4yr and 5-6yr. The twenty does in each age group were then sub-divided randomly into two supplementation groups of ten – the high supplement (HS) and the low supplement (LS). This resulted in four treatment groups in a completely randomized block design.

3.2.2 Experimental Set-up

3.2.2-1 Experimental Supplement Feed Preparation

The supplement feed consisted of 36.4% brewers spent grain (dry), 18.2% dry cassava (kokonte), 22.7% palm kernel cake and 22.7% soya bean meal. Apart from the dry cassava (which was purchased from open market) the other feed stuffs were old stocks of the station. After pounding the dry cassava into coarse flour it was thoroughly mixed with the other stuffs to compound the supplement feed. The chemical composition of the feed stuffs, supplement feed and samples of the main basal forage were determined by proximate analyses at the KNUST Animal Science Department.

3.2.2-2 Dietary Treatment

The six weeks flushing period coincided with the end of the dry season and the beginning of the wet. The rainfall promoted early vegetation re-growth resulting in available basal forage in a 3-ha paddock of *Panicum maximum* (Guinea grass) (Fig.3.4).

Fig.3.4. Re-growing tuft of old *Panicum maximum* plant

Fig.3.5 Experimental animals entering a paddock for grazing

Fig.3.6 Experimental animals being fed individually with supplement

Other grass species especially tufts of *Sporobolus pyramidalis* and old runners of *Cyanodon plectostachyus* were also found in the *Panicum maximum* paddock making the paddock not a pure stand. The experimental animals were grazed (Fig. 3.5) on this fenced paddock during the flushing period from 0900h to 1600h daily. Upon return from grazing, the does were tethered in two roofed dwarf-walled pens, with concrete floors, but without bedding. They were individually provided with the locally made supplement (Fig. 3.6) overnight. Water was supplied *ad libitum* and the animals had access to salt licks placed at vantage points for the use of the entire flock at the Station.

3.2.2-3 Estrus Synchronization

In order to limit time for coming into estrus so as to schedule blood sampling, detection of estrus, mating, and parturition within a pre-determined period estrus was induced and synchronized chemically with Estrumate injection (Schering-Plough Animal Health; NJ). Estrumate (Cloprostenol sodium) is a synthetic prostaglandin analogue structurally related to prostaglandin $F_{2\alpha,}$ containing 250μg/ml of cloprostenol and 0.1% $^{v}/_{w}$ chlorocresol BP as a bactericide. Each doe was injected intramuscularly with 0.25ml (62.5μg cloprostenol) at 0900h on days 33 and 44 of flushing – 11 days apart – to let estrus coincide with end of supplementation.

3.3.0 Data Collection

3.3.1 Supplement Intake

The HS group received 450g/doe/day for 45 days and the LS group, 150g/doe/day for the first 28 days only at 1600h each day on return from grazing. Every morning at 0800h left over feed was weighed and the intake determined by difference. Troughs were cleaned before supplying new feed.

3.3.2 Body Weights and Condition Scores

Body measurements were taken at 0800h on measuring days before animals went for grazing to reduce effect of gut-fill on body weights. A lamb weighbridge (Salter, C & H Engineering; England) was used to weigh the animals on day one and, thereafter, weekly throughout the flushing period. Kids were also weighed within 24h of birth. Similarly, the body condition of the does was scored three times (on days one, thirty-three and forty-five) during the period of flushing. The method of scoring was the six-point system described by Jefferies (1961). It involved a palpating assessment of the degree of fatness/leanness of a goat by describing precisely the feel of sharpness of the edges of the spinal and transverse processes as well as the fill of the eye-muscle of the lumber vertebrae around the loin area immediately behind the last rib and above the kidney (Fig.3.7).

Fig.3.7 Assessing the body condition of a doe

Agreed subjective grade scores were given ranging from zero to five. The main features of the awarding system are as follows:

Grade 0: Very Lean

Extremely emaciated, and on the point of death. Individual dorsal spines are pointed to the touch. Hips, pins, and ribs are prominent. Transverse processes are visible individually. No detectable muscular or fatty tissues are between the skin and the bone.

Grade 1: Lean

Prominent and sharp, are the spines and transverse processes. Fingers pass easily under end of transverse processes. Eye muscle is shallow and without fat cover.

Grade 2: Moderate

Spines and transverse processes are still prominent but somewhat smooth with a feel as fine corrugations. Fingers pass with a little pressure under ends of transverse processes. Eye muscles are of moderate depth but have little fat cover.

Grade 3: Good

The spinous processes have a small elevation, are smooth and rounded, and individual bones can be felt only with pressure. The transverse processes are smooth and well covered, and firm pressure required feeling over the ends. The eye muscles are full and have a moderate degree of fat cover.

Grade 4: Fat

The spinous process can just be detected with pressure as a hard line between the fat-covered eye muscles. The ends of the transverse processes cannot be felt. The eye muscles are full and have thick covering of fat.

Grade 5: Very Fat

The spinous and transverse processes cannot be detected even with firm pressure. There is depression between the layers of fat in the position where the spinous processes would normally be felt. The eye muscles are very full with very thick fat cover. There may be large deposits of fat over the rump and tail.

3.3.3 Detection of Estrus, Mating and Kidding

Thirty one (31) hours after the second Estrumate injection (i.e. at 1600h on day 45 of flushing) supplementation was stopped, does confined in one pen and two of six bucks of known fertility which were already used to one another were introduced to detect early estrus and to mate. Estrus was observed hourly for 72 hours (with confined does supplied with cut forage, water and salt lick *ad libitum*), after which it was continued in the paddock at 6-hour intervals for 48 hours. A doe was confirmed to be in estrus when it willingly stood still and to be mounted by a buck for mating. Mating itself was taken as successful when the buck gained vaginal intromission of the penis, and both doe and buck suddenly arched dorsally as if in response to a ‘painful’ stimulus. During the penned mating, successfully mated does were removed into another pen with the second pair of fresher bucks for extra servicing as long as there was estrus. After every 12 hours, the first two bucks were retired to rest and replaced by the fresher third pair to ensure effective estrus detection and booster mating. All six bucks moved later together with the does when they were re-grouped on the paddock, for further detection of late estrus, return to service and mating. Due to housing problems, the experimental animals re-joined the larger group of expectant does on the 6th day after start of estrus/mating monitoring for grazing and general husbandry care together until parturition. Does kidded on paddocks without any interventions; and kidding was observed twice daily (0800h and 1600h) for 30 days.

3.3.4 Blood Sample Collection

Blood samples were collected (Fig. 3.8) on days 1, 33 and 45 for determination of serum glucose, total protein and calcium as well as the hormones insulin, LH and FSH. On day 1 blood was collected from a total of 8 does (2 randomly picked from each treatment group). On day 33 a total of 20 does (5 from each treatment group) were randomly selected for the blood sample collections, and on day 45 all 40 animals were sampled. About 1.0ml blood was harvested by jugular venipuncture into 1.5ml polypropylene Eppendorf tubes at 0800h before animals went out to graze on days 1 and 33, resulting in a total of 28 samples for the two samplings. On day 45 sampling started at 1600h, after grazing and the last supplementation, and continued at 6h intervals for 24h, giving 5 samples per doe and a total of 200 samples at mating, between 31 and 69 h (inclusive) after the second $PGF_{2\alpha}$ administration. Thus overall 228 blood samples were collected, chilled immediately on ice and then centrifuged at 3000 rpm for 10 minutes using Eppendorf Centrifuge 5702 (www.eppendorf.com/ltd) (Fig.3.9). The sera collected were stored at minus 20°C until later assayed for chemistry and hormones at the laboratory of Kintampo Health Research Centre (www.ghana-khrc.org).

Fig.3.8 Collection of Blood Samples

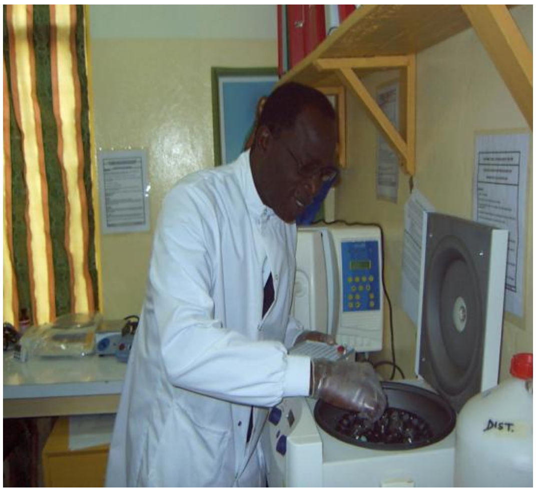


Fig. 3.9 Centrifugation of Blood samples

3.4.0 Analytical Methods

3.4.1 Serum Chemistry Analysis

The concentrations of glucose, total protein, and calcium in the goat sera from blood samples collected before does went out to graze (i.e. 0800h) on days 1 and 33, and 0400h on day 45 of flushing were analyzed, using kits for human serum, and Vitalab Selectra E Chemistry Analyzer (with software version June 2003), Netherlands (www.vital.nl). The kit for each test was equipped with a reaction principle insert and standard curve solutions, provided by Human Biochemistry & Diagnosis Company, Germany (www.human.de).

(a) **Glucose**

The method used was the enzymatic colorimetric test or Glucose-Oxidase Per-Aminophenazone (GOD-PAP) method described by Barham and Trinder (1972). The reaction principle is that glucose is determined after enzymatic oxidation in the presence of glucose-oxidase. The hydrogen peroxide produced reacts under catalysis of peroxide with phenol and 4-aminophenazone to red-violet quinoneimine dye as indicator, with absorbance proportional to the concentration of the glucose in the sample.

$$\text{Glucose} + O_2 + H_2O \longrightarrow \text{GOD} \quad \text{gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{POD}} \text{quinoneimine} + H_2O$$

For this reaction, the kit included a ready-to-use (RTU) enzyme reagent (100ml) and standard glucose solution (3ml).

(b) **Total Protein**

The Biuret method or Photometric Colorimetric Test for total proteins (Weicheselbaum, 1946) was used. Cupric ions react with protein in alkaline solution to form a purple complex. The absorbance of this complex is proportional to the protein concentration in the sample. The kit comprised four vials (100ml each) of color reagent (sodium hydroxide, potassium sodium tartrate, copper sulphate and potassium iodide) and one vial (3ml) of standard protein – all RTU.

(c) **Calcium**

This followed the Photometric Test – Cresolphthalein-complexone (CPC) method (Gitelman, 1967). Calcium ions react with O-Cresolphthalein-complexone in alkaline medium to form a purple colored complex. Inclusive in the kit were a 100ml buffer solution, 100ml color reagent and 3ml standard solution of calcium. The working reagent was prepared by mixing equal volumes of the buffer solution and color reagent (Fig.3.10). The mixture was allowed to stand for 30 minutes at room temperature.

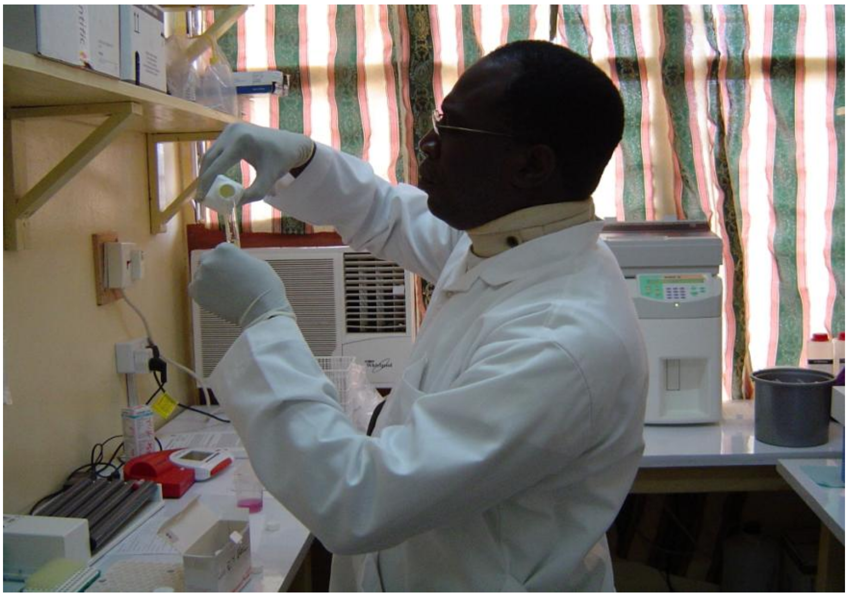


Fig.3.10 Preparation of the calcium test working reagent

The chemistry analyzer works on the principle that the light absorbance (or optical density) of a colored end-product of a biochemical reaction is directly proportional to concentration of the reactant. It automatically converts the absorbance into concentration from a standard curve of absorbance against concentration. The analyzer is, therefore, programmed to automatically:

- Identify the locations of reagent bottles and labeled tubes for samples, standard and quality control solutions.
- pipette the right volumes of reagent and reactant (analyte) solutions
- Mix reactants and reagent solutions in semi-micro cuvettes and/or incubate at set temperature and time.
- Read the light absorbance of colored end-products at given wavelengths; calibrate and construct the standard curve
- Convert the absorbance to reactant (analyte) concentration
- Perform statistical calculations as required/instructed.

Accordingly, the same procedure was followed in the analyses of the goat serum chemistry:

- The analyzer was programmed with the pipetting, incubation and absorbance scheme in Table 3.2.
- Reagent bottles and sample cups were labeled and filled with their appropriate solutions
- The filled reagent bottles and sample cups were loaded on the analyzer (Fig.3.11) according to the computerized locations displayed on the monitor (Fig. 3.12). Quality controls were replicated.
- A click to confirm loading started the operation of the machine to perform all 3 tests on each sample loaded.
- The displayed results were copied from the monitor.

Fig.3.11 Reagent bottles and sample cups arranged in analyzer according to their displayed locations on monitor

Fig 3.12 Displayed locations of reagent bottles and sample cups on monitor

Table 3.2: Pipetting, Incubation and Filtration Scheme for the Goat Serum Chemistry Analysis

TEST SOLUTION	VOLUME PIPETTED (µL)	INCUBATION		LIGHT FILTRATION		ABSORBANCE READING TIME (min)
		TEMP (°C)	DURATION (MIN)	WAVELENGTH (nm)	OPTIC PATH (cm)	
Sample for Glucose	3.0	37.0	5.0	500	1.0	5.0
Sample for Total Protein	3.0	37.0	5.0	540	1.0	5.0
Sample for Calcium	7.0	-	-	570	1.0	5.0
Standards	3.0	As for sample	As for sample	As for sample	1.0	5.0
Controls	3.0	As for sample	As for sample	As for sample	1.0	5.0
Reagents	300.0	-	-	-	-	-

3.4.2 Serum Hormones Analyses

Enzyme Linked Immunosorbent Assay (ELISA) kits (Calbiotech, USA) and ELISA Reader (Dynextech, USA) designed for hormones in human serum/plasma, were used in the study. ELISA is a solid phase two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the antigen (hormone).

The principle may be outlined as follows (Fig. 3.13):

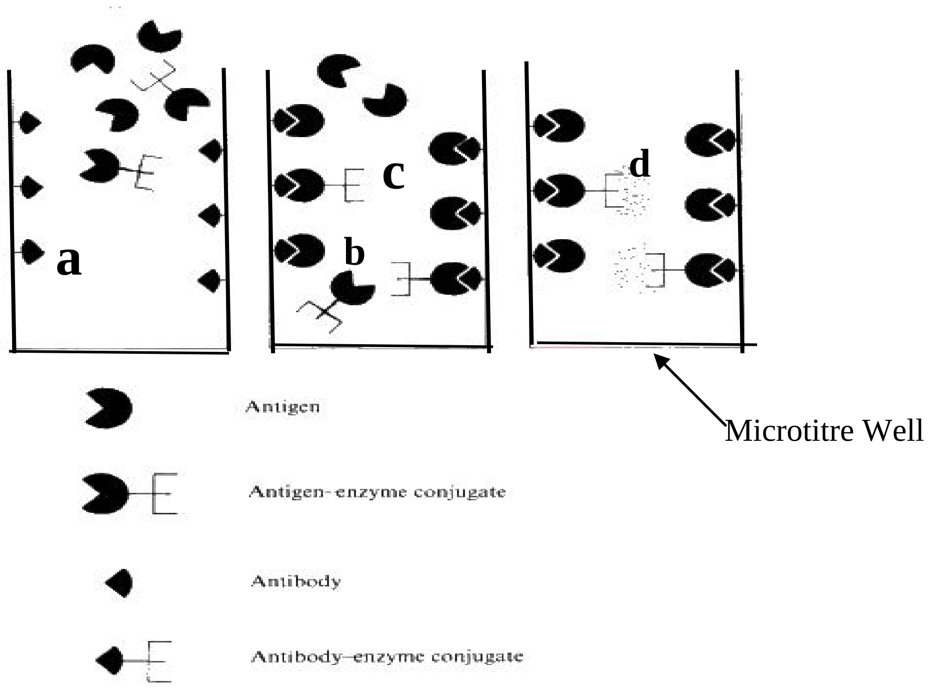


Figure 3.13 The Principle of ELISA (Labels on figures are explained below)
Source: Immunocensors http://www.Isbu.ac.uk/biology/enztech/immuno.html
[Accessed 2006, 20 June]

a. Anti-hormone antibody is fixed to microtitration well

b. Hormone being assayed in sample reacts with and is bound to the fixed anti-hormone antibody at one surface

c. Biotin-conjugated anti-hormone antibody reacts with the bound hormone on the other surface and thereby sandwiching the hormone between the two anti-hormone antibodies

d. Streptavidin Peroxide Enzyme Complex binds to the biotinylated anti-hormone antibody with a biotin-avidin affinity

e. The bound enzyme complex is detected by reaction with a substrate, which gives a colorimetric endpoint that is read spectrophotometrically.

The ELISA READER is a computerized spectrophotometric device, programmed to automatically convert light absorbance of a colored biochemical end-product to the concentration of the reactant (analyte) based on a validated proportionality (standard) curve of absorbance versus concentration. A maximum of 96 tests (samples, standards, and controls) can be read within seconds in an assay, from 96 microtitre wells on a holder (Fig 3.14). The results are immediately displayed on a monitor and can be printed.

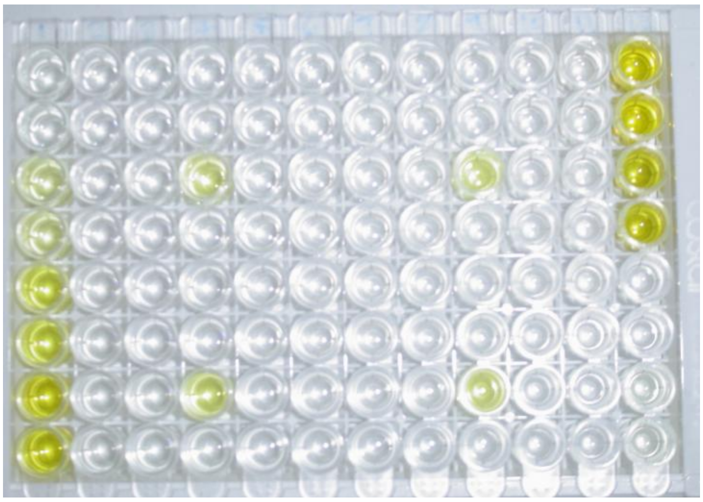


Fig 3.14 Incubated sera samples, standards and controls in ELISA microtitre wells for the FSH assay in this study.

The yellow coloration indicates a reaction and detection of FSH. The wells in the extreme column (left) are the standards and those in the twelfth column (right) are human sera controls. Columns 2-11 represent the wells for the goat sera samples. The four colored wells in this region are for samples of doe # 0375 collected 31, 37, 43 and 69h afterPGF$_{2a}$ *second injection in wells (4,3), (9,3), (4,7) and (9,7), respectively across the plate (L-R). The intensity of the coloration is reflection of the concentration of FSH in the serum*

The ELISA Reader used for the three hormones (LH, FSH, and Insulin) in this study was MRX Revelation ELISA Microplate Reader 4.22, with 2001 version of software from Dynex Technology, USA (www.dynextechnologies.com).

For the assay the frozen specimen from only blood samples collected on day 45 of flushing were used. The samples were thawed at room temperature and homogenized with vortex. Each hormone was assayed with its own kit and procedure, after both samples and reagents settled at room temperature. The kits were supplied from CALBIOTECH INC., California, USA (www.calbiotech.com).

3.4.2-1 Luteinizing Hormone (LH):

The LH kit (from CALBIOTECH, California) was equipped with the following materials:

1. Microwells coated with LH Mab (anti-LH antibody), 12 strips x 8 wells (96 wells)
2. LH Standards: 6 vials (RTU), with concentration of 0, 10, 20, 40, 80, 140mIU/mL covering the physiological range of humans, 0.7ml

3. LH Enzyme conjugate (HRP): 1 bottle (RTU), 12.0ml
4. TMB substrate: 1bottle (RTU), 12.0ml
5. Stop solution: 1 bottle (RTU), 12.0ml
6. 20x Wash concentrate:1 bottle, 25.0ml

The assaying steps were as follows:

a) A 500ml washing buffer solution (1x) was prepared by mixing the 25ml wash concentrate (20x) to 475ml distilled water and left at room temperature.
b) A labeling spreadsheet was prepared to locate positions of the samples, standards, and controls corresponding in turns to that of the Reader and arrangement of the micro titre wells respectively.
c) Using precision pipettes and disposable tips, 50µL of LH standards, the goat sera samples and a human serum sample (for quality control) were placed inside the microtitre wells at the labeled positions. Only the standards and controls were replicated.
d) 100µL of enzyme conjugate was added to each of the wells
e) The microwell plate was covered with adhesive strips and incubated for 30 minutes at room temperature.
f) The wells were emptied of their liquid contents and washed 3 times with 300µL of the 1x wash buffer; each wash lasting about 30 seconds. Absorbent paper towel was used to blot off the wash buffer from the wells.
g) 100µL of TMB substrate was added to all wells, and incubated for 10 minutes at room temperature.
h) The reaction was halted by adding 50µL of the stop solution as the plate was shaken gently to mix the solution.
i) The absorbance was read immediately (within 5 minutes of stopping the reaction) on the ELISA Reader at 450nm filtration.

3.4.2-2 Follicle Stimulating Hormone (FSH):

The FSH concentration was determined with the same procedure as for LH. The FSH kit contained also similar materials and quantities as for LH, except that:

- The micro wells were coated with FSH Mab (anti-FSH antibody)
- The FSH standards have concentrations of 0, 15, 30, 60, 120, and 240 mIU/mL; covering the physiological range for humans.

3.4.2-3 INSULIN:

The kit for insulin assay provided the following materials:

1. 1 plate of 96 microwells coated with anti-insulin antibody (RTU)
2. Five vials each containing 1.0ml of standard insulin with concentrations of 6.25, 12.5, 25, 50, and 100µIU/mL (RTU), which covers human physiological range.
3. 1 vial of 3.0ml standard 0, (RTU)
4. 1 vial of 3.5ml (RTU) anti-insulin biotinylated conjugate
5. 1 vial of 7.0ml (RTU) Streptavidin HRP (enzyme) complex
6. 1 bottle of 30ml 40x concentrated washing solution to be reconstituted
7. 1 vial of 14ml (RTU) Peroxidase substrate (TMB), which was noted to be light sensitive
8. 1 vial of 14ml (RTU) stop solution, (0.5M H_2SO_4)

The assay was done with the following procedure:

(a) A 1.2L washing solution (1x) was prepared by mixing the 30ml (40x concentrated) wash with 1170ml distilled water

(b) A location identification spread sheet was prepared in line with the microplate and Reader

(c) 25µL of insulin standards, control (human serum) and the goat sera samples were pipetted using precision pipettes with disposable tips, into the microwells according to the locations on the spreadsheet.

(d) 25µL of anti-insulin biotinylated conjugate was added to all wells, and incubated for 30 minutes at room temperature (RT)

(e) The wells were emptied of liquid content and washed 3 times with 350µl washing solution to each well. The wash was completely aspirated with absorbent paper towel.

(f) 50µl of Enzyme complex (HRP) was added to each well and incubated for 30 minutes at RT

(g) Another washing was done 3 times as described above

(h) Then 50µl of the peroxide substrate (TMB) was added to the wells and incubated for 15 minutes at RT

(i) 50µL of stop solution was added and the absorbance matrix and corresponding concentrations read at 450nm.

3.4.3 Analysis of Supplement and Forage

Proximate analyses of the supplement and forage feed used in the study were done at the Department of Animal Science Laboratory, Kwame Nkrumah University of Science and Technology (KNUST). The parameters analyzed were, Dry Matter (DM), Crude fat or Ether Extract (EE), Crude Fiber (CF), Crude Protein (CP) and Ash. Nitrogen free extract (NFE) was determined by difference. All analyses of feed followed methods described by Nahm (1992). Calcium was also analyzed spectrophotometrically at the Medical Laboratory of Okomfo Anokye Teaching Hospital (KATH), Kumasi.

The data on physical body measurements, serum chemistry, hormone assays and litter size at birth were statistically analyzed for treatment differences using Least Significance Difference (LSD). Relationships were determined by regression and correlation analyses. All the statistical procedures followed methods described by Bailey (1995).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1.0 Chemical and Energy Components of the Experimental Forage and Concentrate Supplement

The chemical composition of the basal forage species (*Panicum maximum*) and the concentrate supplement feed are shown in Table 4.1.

Table 4.1Chemical Analyses (DM %) of feed stuffs, Supplement feed and Forage

Feed Stuff	%DM	%CP	%CF	%EE	%Ash	%NFE	%Ca	MEMJ/Kg[1]
Dry brewers' grain	98.83	20.3	17.40	5.10	7.40	55.10	0.27	11.22
Dry coarse cassava flour	95.85	1.2	2.00	0.30	0.20	96.30	0.11	13.31
Palm kernel cake	86.38	29.5	15.60	5.12	12.30	37.48	0.30	12.47
Soya bean meal	90.22	49.9	5.10	3.00	11.20	30.90	0.30	13.31
Supplement Feed	93.50	23.10	11.0	5.60	6.00	54.30	0.31	12.30
Forage *Panicum maximum*	29.40	9.70	27.29	2.60	19.30	41.11	0.40	8.75

1. ME (MJ/kg) values for feed stuff were book values (Devendra and McLeroy, 1992), from which that for the supplement was calculated.

The proximate composition of the grazer forage suggests that it was nutritionally adequate to meet the estimated protein and energy requirements of 36.0g (or 6.0% DMI) crude protein and 5.50MJ ME per day for does with average weight of 17.8kg and growing at an average rate of 36.66g/day (Devendra and McLeroy, 1992; Peacock, 1996) if there were abundant lush and if the animals could eat at least 3% of their body weight of dry matter (DM) (Steel, 1996).

It also appears that the concentrate supplement was rich enough to meet the energy (6-13MJ/KgDM ME: Steel, 1996) and digestible crude protein (16 – 18%: Steel, 1996) concentrations in typical goat feed as well as the requirement of does for flushing (Nix, 2004).

4.2.0 Basal Forage and Supplement Dry Matter Intake

It was observed that the animals readily and selectively grazed on the *Panicum maximum* forage during the flushing period. This may suggest high palatability due to its relatively low crude fiber content (27.29%). Goats are selective feeders and given the opportunity they tend to feed more on non-fibrous species (Peacock, 1996).

Although not measured, the dry matter intake of the basal forage during the flushing period might, perhaps, be less than expected because of reduction in grazing hours. It was observed that the animals often stopped grazing about an hour before time of supplementation during the first half of the period, especially after the second week; and were also interrupted by rainfall during the second half. According to Steel (1996) goats will stop grazing if disturbed by rain. The early stoppage of grazing might be a psychological response to boredom especially resulting from a self-imposed regulation of feed intake as lipostatic reaction to sudden increase in plane of nutrition (McDonald *et al,* 1998). It could also be in anticipation of the supplement feed due to insufficient satiation on the pasture (Peacock, 1996) or greater desire for the newly introduced and more nutritious concentrate being fed at a known time (1600h) than for the forage. Animals supplied with both grazer or fodder and concentrate supplement tend to replace the coarse fodder with the more expensive supplement instead of adding it (Chesworth, 1992).

The mean (±SD) daily dry matter intake of the supplement feed is shown in Table 4.2. Paired comparison analysis of variance showed that both within and between HS and LS groups the differences in means were significant ($P < 0.05$).

This was expectedly due to the quantity supplied (450/doe/d v 150/d/day, respectively). An average of 20.64g and 7.23g per kg of body weight were eaten as supplemented DM in the HS and LS groups, respectively. With total DM intake requirement of tropical goats being between 2.5 and 3 % body weight (Devendra and McLeroy, 1992; Steel, 1996; Peacock, 1996) the supplement contributed only about 20.79% in the LS group but 60.09% for the HS group.

Table 4.2 The Main Effects of Supplementation level and Age on Supplement Dry Matter Intake by Flushed Does.

Supplement dry matter intake	High Supplement: (450g/d x 45d)		Low Supplement: (150g/d x 28d)	
	3-4yr	5-6yr	3-4yr	5-6yr
Mean feed intake (g)	359.14±32.36	373±22.12	124.5±1.56	125.2±0.0
Mean body weight(kg)	16.95±2.2	19.08±2.0	16.19±1.57	18.76±1.69
Mean Feed intake per body weight(g/kgBW)	21.19±1.94^{a}	19.57±1.80^{b}	7.69±0.09^{c}	6.67±0.00^{d}
Percent of daily requirement	59.80%	62.10%	20.75%	20.93%
Main treatment values	20.64±2.86g/kgBW^{a} 60.09%		7.23±0.88g/kgBW^{b} 20.790%	

Means in the same rows with different superscripts are significantly different (P<0.05)

The 3-4yr does showed higher (P<0.05) mean DM intake of 21.19±1.94g at high supplementation and 7.69±0.09g at low supplementation than the 5-6yr does with corresponding values of 19.57±1.80g and 6.67±0.00g per body weight, respectively. The 3-4yr goats ate more than the 5-6yr ones because they were, perhaps, more distressed nutritionally and with a lower than average body weight of 14.69±1.73kg needed more nutrients for compensatory growth (McDonald, 1998) than the 5-6yr olds that weighed 17.5 0±2.46kg) prior to the experiment. The minimum flock average body weight at the station was 16.5kg (range of 15-18kg). The possible nutritional distress might be as a result of dry season feed scarcity and prolonged lactation just before the experiment. In such case the lighter and younger animals experience greater distress (Mtimuni, 1995).

4.3.0 Effects of Flushing on Physical Body Status of Does.

Tables 4.3 and 4.4 show the mean gains (±SD) and percentage gains in body weight and condition score (BCS) at mating due to main effects and interactions of treatment (supplementation level and age), respectively, in the experimental does. For main treatment effects, the high supplemented (HS) and low supplemented (LS) does had mean initial (16.28±2.42 and 15.91±2.69kg, respectively), and final (18.01±2.35 and 17.49±2.06kg, respectively) body weights resulting in non-significantly different ($P>0.05$) mean weight gains of 1.73±1.18 and 1.58±1.29kg, respectively (Table 4.3; Fig 4.4). The corresponding values for the 3-4yr and older 5-6yr does were 14.69±1.73 v 17.50±2.46kg and16.58±1.92 v 18.92±1.84kg with the 3-4yr does gaining an average of 0.47kg more ($P<0.05$) than the 5-6yr ones. The average daily gains tended to be higher in the HS than LS (38.44 v 35.11g/day) and for the 3-4yr than 5-6yr (42.00 v 31.55g/day). These main treatment effects moved the entire flock mean initial body weight from 16.09±2.53kg to final mean value of 17.75±2.20kg, with mean weight gain of 1.65±1.23kg (or 10.25%) and average apparent growth rate of 36.66g/day.

Body weight gain (1.89±1.06kg or 13.26%) of the 3-4 year does in low supplement group (Table 4.4) was statistically same ($P>0.05$) as the gains (1.88±1.35kg or 12.48%) for the counterparts in the high supplemented group. In the 5-6yr does the LS group gained 1.26±1.69kg, which was lower ($P<0.05$) than for the HS (1.58±1.03kg).

Table 4.3 Main Treatment (Supplement Level and Age) Effects on the Dry Matter Intake and Physical Measurements at Mating of Flushed Does.

Main Treatments	Supplement level		Age	
Parameters	High supplemented does	Low supplemented does	3-4yr does	5-6yr does
Mean supplement dry matter intake (g/kg BW)	20.64±2.86^{a} (60.09%)	7.23±0.88^{b} (20.80%)	14.87±7.52^{c} (43.37%)	13.43±3.81^{c} (42.35%)
Mean initial body weight (kg)	16.28±2.42	15.91±2.69	14.69±1.73	17.50±2.46
Mean final body weight (kg)	18.01±2.35	17.49±2.06	16.58±1.92	18.92±1.84
Mean body weight gain (kg)	1.73±1.18^{a}	1.58±1.29^{a}	1.89±1.06^{a}	1.42±1.35^{b}
Percent mean weight gain (%)	10.62	8.13	12.86	8.11
Average growth rate (g/day)	38.44	35.11	42.00	31.55
Mean initial body condition score (BCS)	1.70±0.49	1.82±0.51	1.65±0.36	1.87±0.60
Mean final BCS	2.22±0.58	2.14±0.36	2.11±0.40	2.25±0.54
Mean BCS gain	0.52±0. 45^{a}	0.32±0.38^{b}	0.45±0.44^{a}	0.38±0.42^{b}
Percent mean BCS gain	30.58	17.30	27.27	20.32

Means (±SD) in the same row with different superscripts are significantly different ($P<0.05$)

DMI in bracket indicates %daily requirement eaten from supplement feed

Similarly the body condition scores (BCS) of the animals improved from mean initial values (1.70±0.49, 1.82±0.51, 1.65±0.36 and 1.87±0.60) to final scores (2.22±0.58, 2.14±0.36, 2.11±0.40, and 2.25±0.54) in the HS, LS, 3-4yr and 5-6yr does, respectively. This resulted in significantly higher ($P<0.05$) mean gain (0.52±0.45 or 30.58%) in HS than for LS (0.32±0.38 or17.30%), and 0.45±0.44 or 27.27% for 3-4yr than 5-6yr does (0.38±0.42 or 20.32%). The treatment interactions resulted in statistically greater ($P<0.05$) mean score gain of 0.62±0.42 or 37.5% in the HS (3-4yr) group than for the HS (5-6yr), LS (3-4yr) and LS (5-6yr) groups which gained 0.42±0.48, 0.30±0.40, and 0.33±0.38, respectively. With the BCS gains in the latter three treatment combinations not significantly different ($P>0.05$) the result for BCS gains were pooled for the experimental flock.

Table 4.4 Interaction of Supplementation level and Age on Physical Changes in Flushed Does at Mating

Parameters	High supplement		Low supplement	
Physical measurements	3-4yr	5-6yrs	3-4yrs	5-6yrs
Mean initial body weight (kg)	15.07±2.00^{b}	17.50±2.33^{a}	14.27±1.31^{b}	17.50±2.79^{a}
Mean final body weight (kg)	16.95±2.22	19.08±2.06	16.19±.57	18.76±1.69
Mean body weight gain (kg)	1.88±1.35^{a}	1.58±1.03^{b}	1.90±0.76^{a}	1.26±1.69^{c}
Percent mean weight gain	12.48	9.03	13.26	7.20
Mean daily weight gain (g/doe/d)	41.7±30.0^{a}	35.11±22.99^{b}	42.20±16.89^{a}	28.00±36.69^{c}
Mean initial body condition score (BCS)	1.60±0.31	1.80±0.63	1.70±0.42	1.95±0.59
Mean final BCS	2.22±0.52	2.22±0.66	2.00±0.22	2.28±0.42
Mean BCS gain	0.62±0.42^{a}	0.42±0.48^{b}	0.30±0.40^{b}	0.33±0.38^{b}
Percent mean BCS gain	38.75	23.33	17.64	16..90

Means (±SD) in the same rows with different superscripts are significantly different ($P<0.05$)

The mean initial and final BCS for the entire experimental flock were 1.76±0.50 and 2.18±0.47 respectively and a gain of 0.42±0.42 (or 23.86%).

Graphical demonstrations of distribution of the initial and final BCS (Fig. 4.1a, 4.1b, and 4.1c) and body weights (Fig.4.2a, 4.2b, and 4.2c) in the treatment groups and entire experimental flock, before and after flushing, are presented. Supplementation level tended to skew the body measurements from mono- to bimodal values after flushing The pre-flushing flock modal BCS was skewed from 1.5 to bimodal scores of 2.0 and 3.0 (Fig. 4.1c) and live weight range from 13.0-17.0kg to bimodal live weight ranges of 13-15kg and 17-19kg (Fig 4.2c). Three does in the lower modal range and one in the higher modal range consistently displayed anorexigenic characteristics of reduced appetite and weight decline, during the second half of the flushing period till death in about 6 – 8 weeks post partum (i.e. after the experiment was long over).

The improvement in physical measurements due to higher supplementation is consistent with the goals of flushing (Nix, 2004) as a static effect in the long term (Cox *et al.,* 1987). That at mating BCS appears to depict gains in physical body improvements than live weight and that the distribution of the physical measurements at mating displayed bimodal values corroborate similar observations in British breeds of sheep (Oppong-Anane, 1975) and Djallonke sheep in Ghana (Blasu, 1981).

BCS assesses leanness or fatness and therefore is more directly related to real metabolic depositions due to improved nutrition (Van Nierkerk, 1982) than body weight. Russell *et al.* (1969) indicated that scores 1.5 and 3.0 (on hexed-scale) are respectively equivalent to 15.7 and 28.8 percent total chemical fat in fleece free empty body of Blackface ewes. Moreover, scores 1-3 (on pento-scale) represent more lean than fat in (Pigmy) goats. Although BC scoring is subjective, the error of subjectivity is appreciably reduced with the use of the same assessor over time; thus a high level of repeatability, both between measurements and assessors, can be obtained (Nicholson and Butterworth, 1986) giving a fair and acceptable assessment of the real nutritionally induced physical status of the animals.

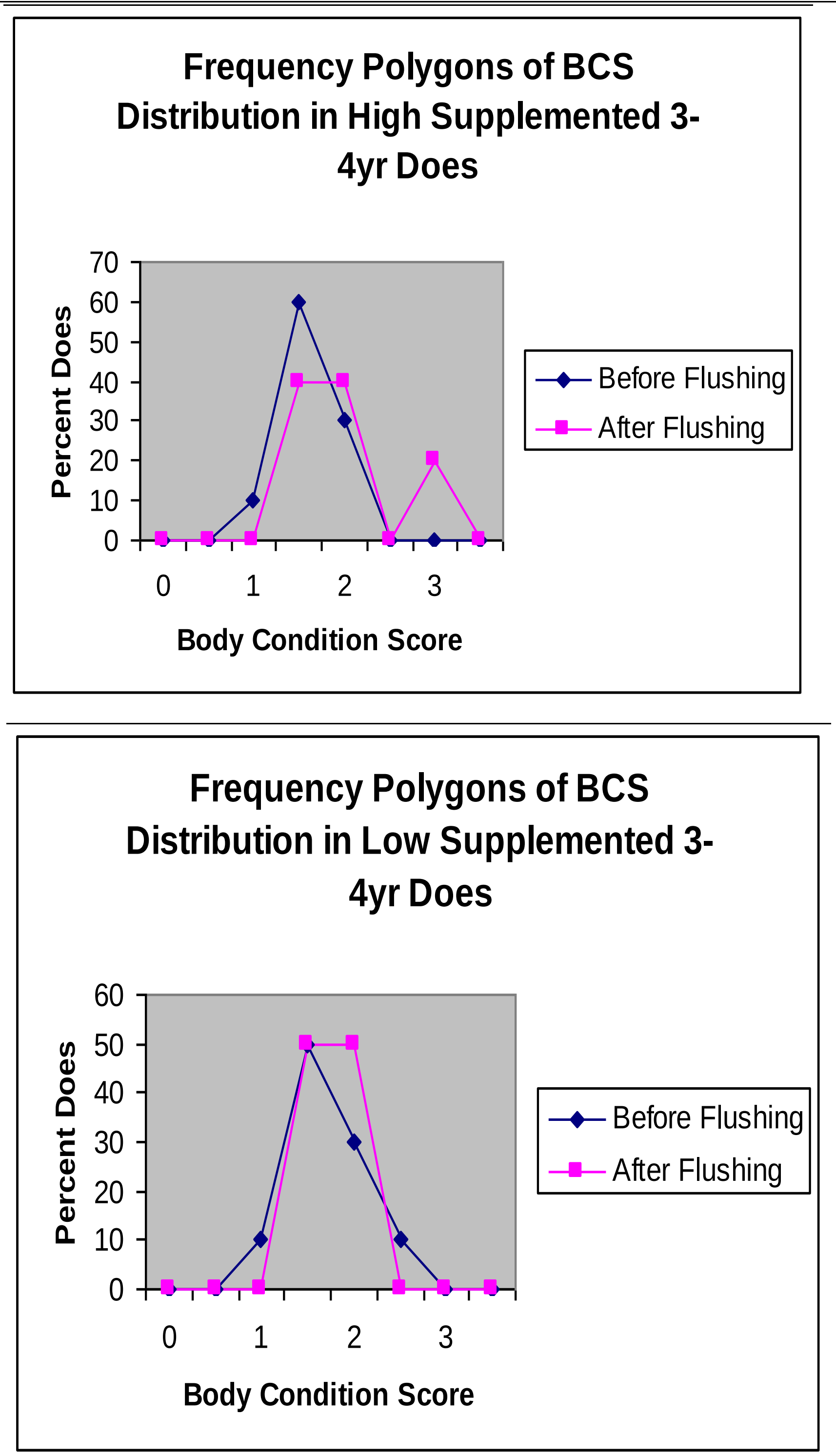


Fig 4.1a Frequency Polygons of the Effect of Supplementation level on Distribution of Body Condition Scores of 3-4yr Does

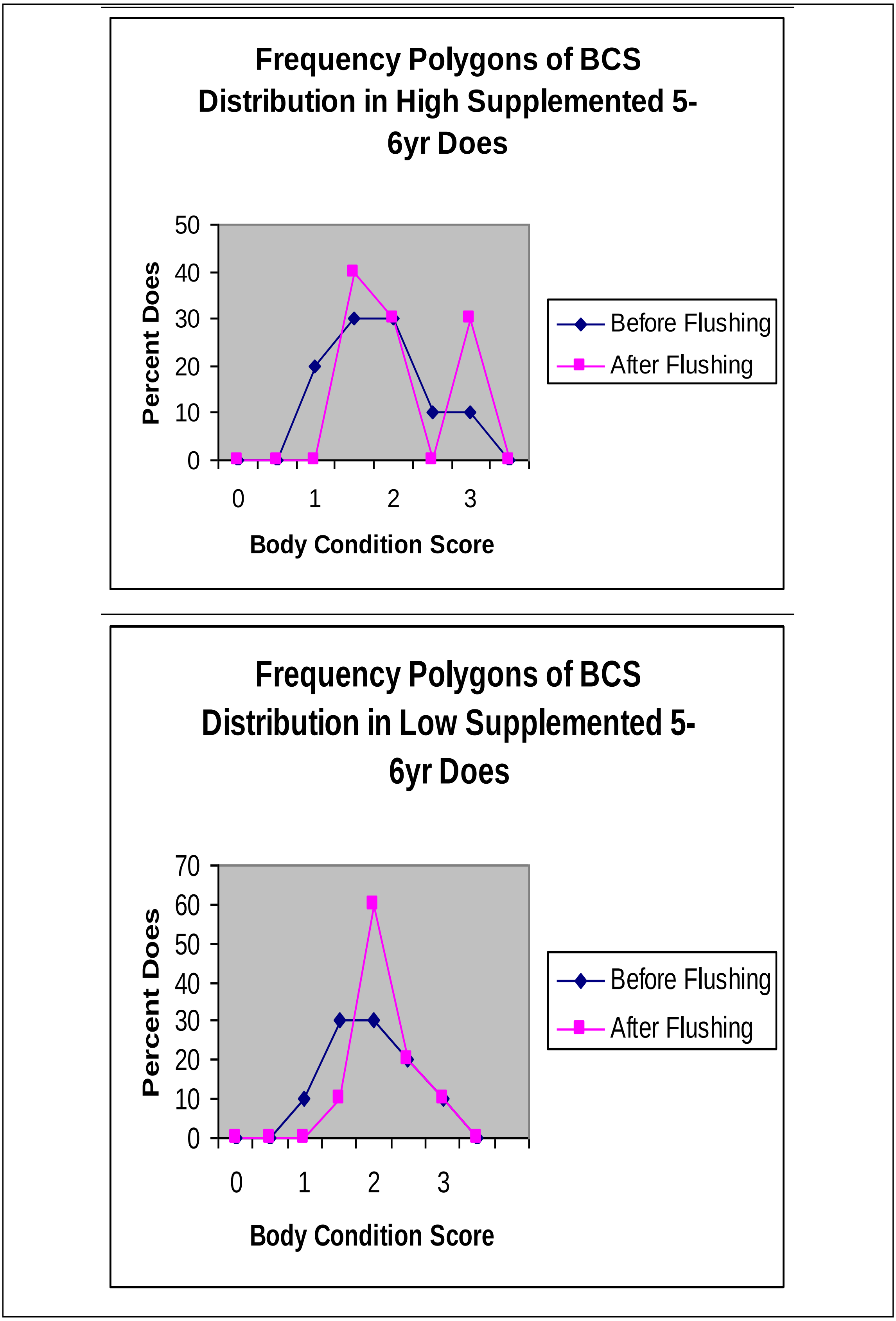


Fig 4.1b Frequency Polygons of the Effect of Supplementation Level on Distribution of Body Condition Scores of 5-6yr Does

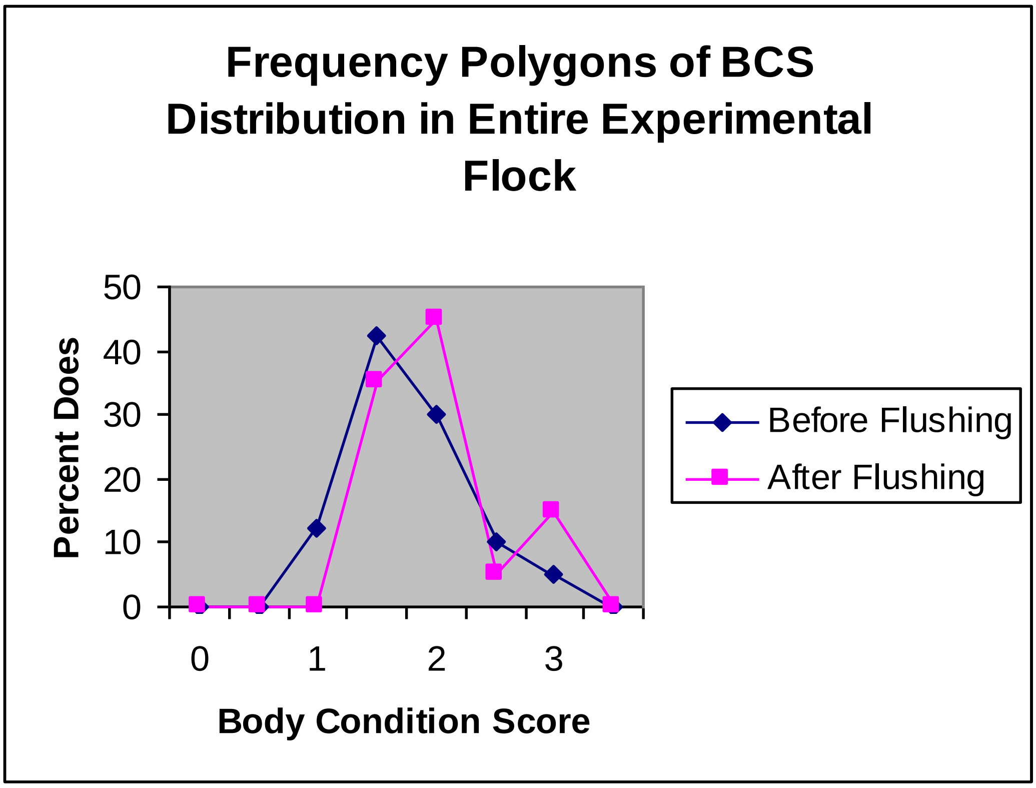


Fig 4.1c Frequency Polygons of the Distribution of Body Condition Scores of Entire Experimental Flock before and after Flushing

The bimodal physical measurements after flushing may be attributed to weight and body score variations in each group, both before and after flushing. Whereas pre-treatment distribution of these physical measurements ranged from 11.5kg to 21.5kg for body weight and 1.5 to 3.5 for BCS, the distribution of growth rates during flushing clustered around two mean ranges: 28 to 35g/day and 41.2 to 42.2g/ day (Table 4.4) in the experimental flock. Consequently after flushing the final physical measurements also tended to be distributed around two means: 2.0 and 3.0 for BCS and mean ranges of 13 to 15kg and 17 to 19kg for body weight. This agrees with the observation of Oliver and Broadbent (1972) cited by Oppong-Anane (1975) who stated that within a flock the range of condition at any one time will unlikely be more than two scores though the optimal range may change with time and season. The tendency for bimodal mean weights was further illustrated by the pattern of the mean weekly weights (or apparent growth) of the animals during flushing.

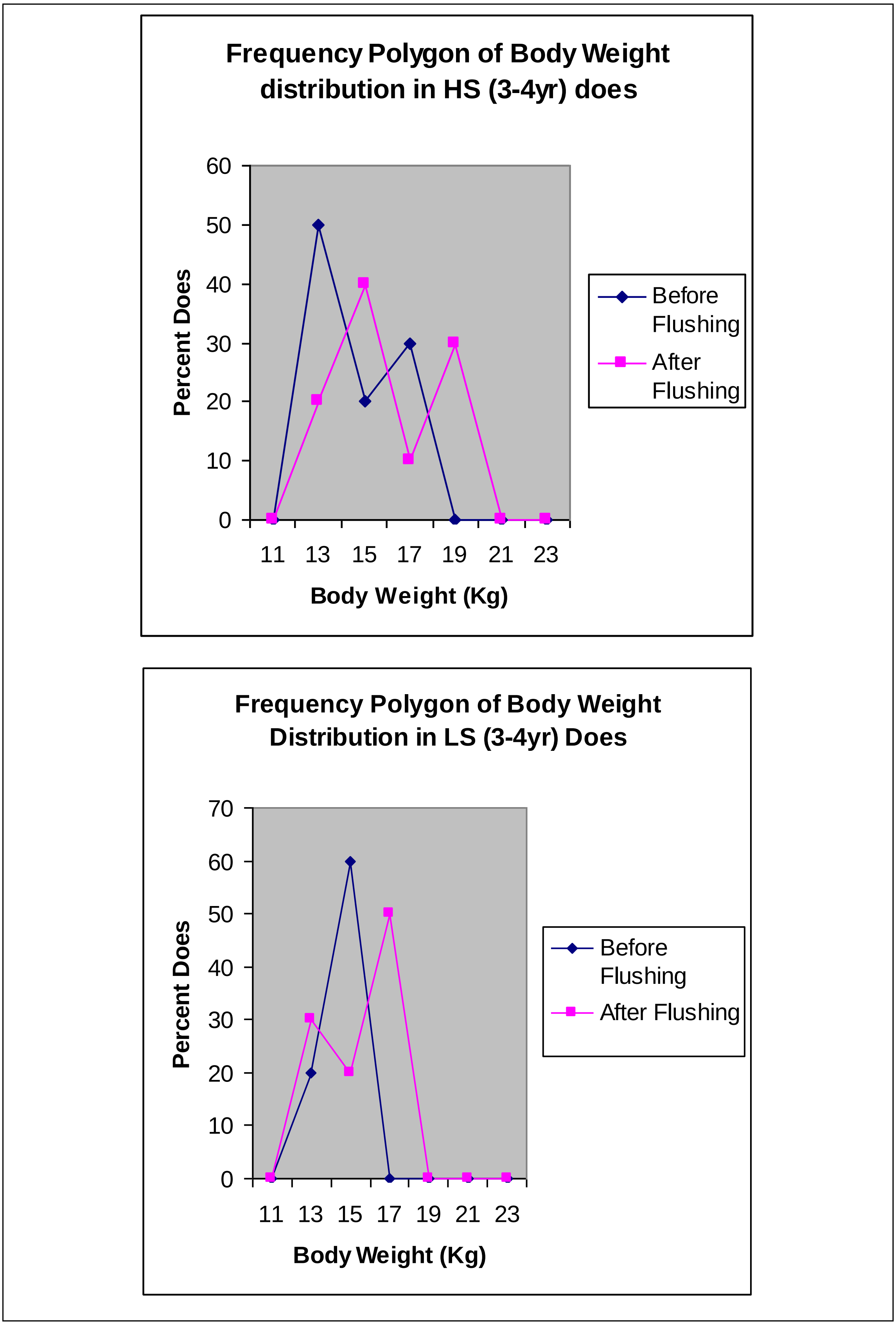


Fig 4.2a Frequency Polygons of the Effect of Supplementation Level on Distribution of Body Weight of 3-4yr Does

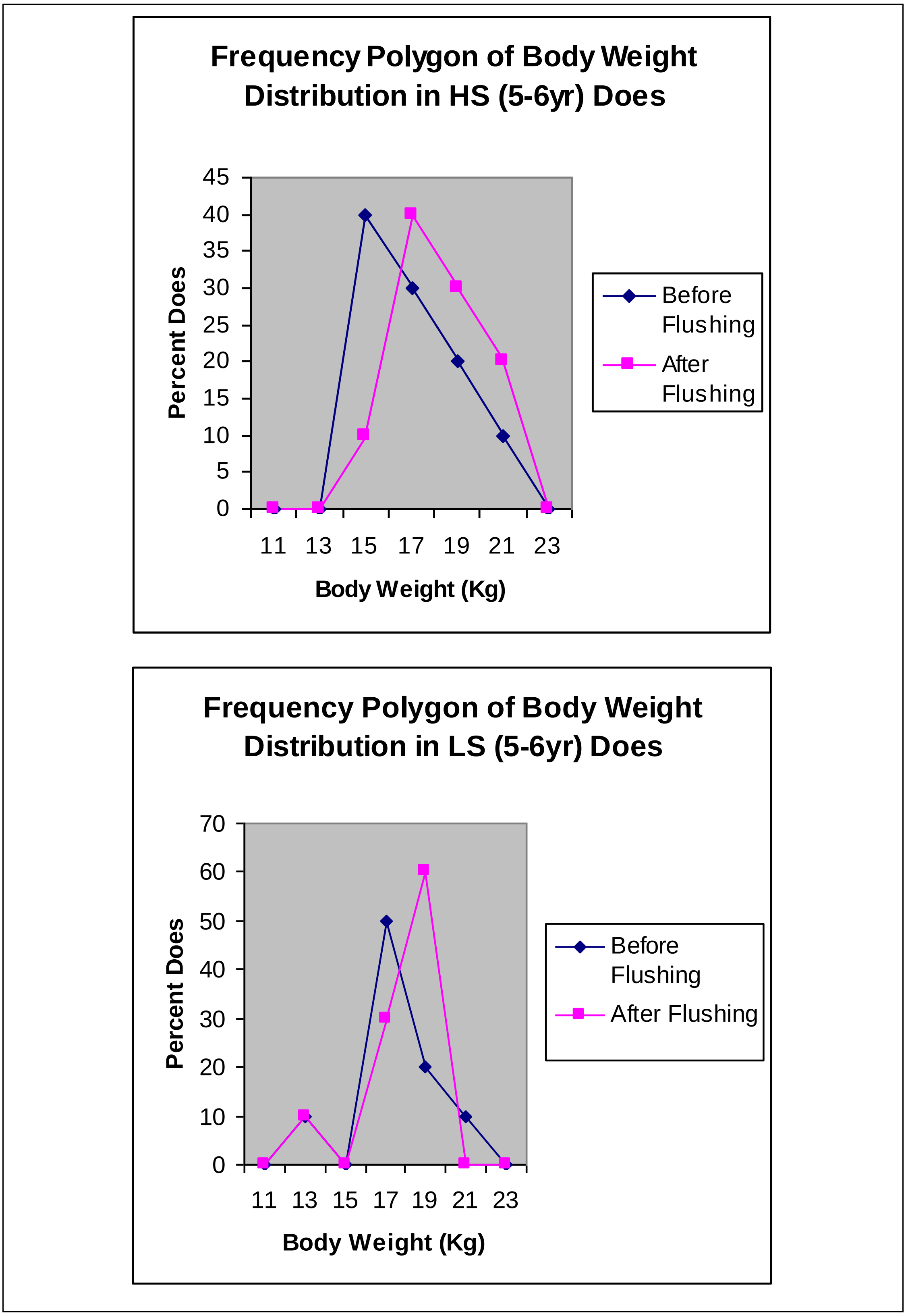


Fig 4.2b Frequency Polygons of the Effect of Supplementation Level on Distribution of Body Weight of 5-6yr Does

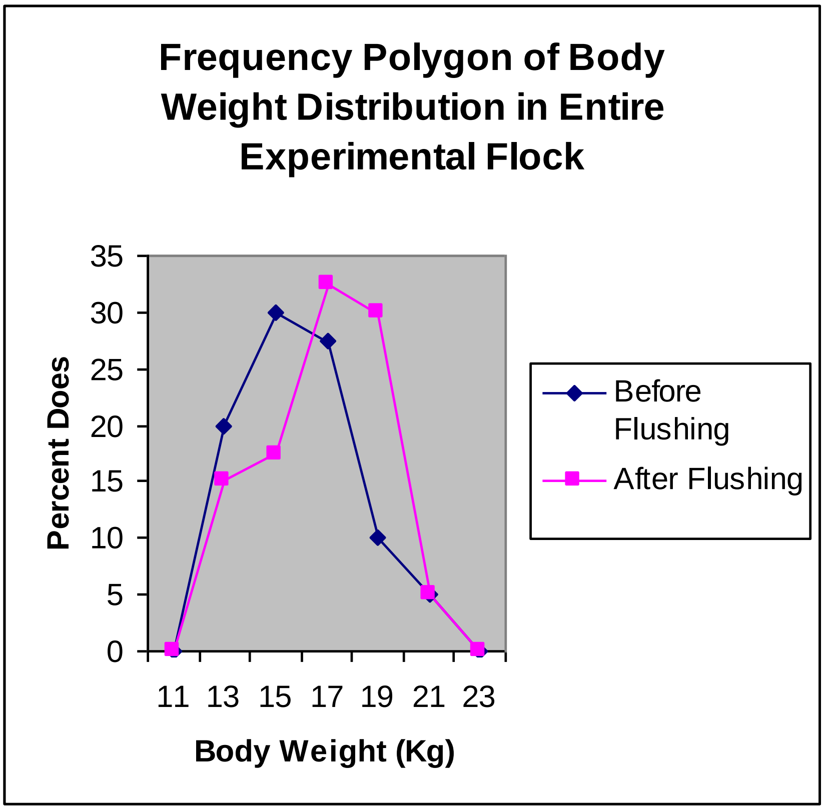


Fig 4.2c Frequency Polygons of the Distribution of Body Weight of Entire Experimental Flock before and after Flushing

The 5-6yr does were heavier than the 3-4yr olds and this difference was kept in their mean weight changes over the period (Fig.4.4).

The percent gains in BCS of 30.58 and 17.30 for HS and LS, respectively are lower than the 57.0% and 39.0% reported for supplemented and non-supplemented Spanish does, respectively (Hart *et al.*, 1999). The discrepancy between the two results may be due to breed and feed differences, because temperate animals are generally bigger and are more efficient in feed utilization than their tropical counterparts (Larenstein bulletin, 1994).

The weight gains due to flushing followed the same pattern as for BCS except that the LS group gained statistically the same weight as the high supplemented counterpart at mating. Similar results were reported for WAD goats in Ghana: Barton and Asiedu (1972) found that non-supplemented goats made weight gains comparable to those for goats grazing and receiving supplement as well; and Blasu (1981) observed that the average percent weight gain in Djallonke ewes supplemented for 3 weeks was not significantly better ($P>0.05$) than that for the non-supplemented group. In the latter case insufficiency of duration (3 weeks) of supplementation and nutrient adequacy of the basal forage grazer (for the non-supplemented group) were incriminated. The current study, however, covered six weeks, but also used a basal grazer with a chemical composition that could adequately meet the estimated requirement of goats. Therefore, while no apparent reason could be given for the lack of significant treatment difference in weight gain between the two groups (HS v LS) of does, nutrient adequacy of the basal forage may be a contributing factor. It is also possible, however, that the pattern of apparent growth rate (daily weight change, Fig 4.3) during flushing was influenced by the animals' tendency to respond to flushing with alternating compensatory weight gain and a desire to maintain minimum (pre-flushing) average body weight. Perhaps, the LS group had a better overall effect of these responses resulting in an almost equal gain in weight as for the HS ones.

The tendency to gain compensatory weight could have resulted from the apparent weight loss before the experiment, possibly, due to the dry season food scarcity and three to four months of lactation. The average weight for 3-6yr does at the end of the rainy season in October 2004 at the Station was 16.5kg (range: 15-18kg). Therefore, the experimental animals had some weight loss by April 2005, with average weight of 15.5kg (range: 14-17kg) just prior to the experiment. The loss in weight during the dry season below the average at the end of the previous rainy season could predispose them to rebound (a more efficient and rapid rate of) metabolism. The rebound metabolism results from increased intracellular activity of enzymes of intermediary metabolism and increased sensitivity to insulin–enhanced cellular uptake and utilization of glucose and amino acids) due to flushing (Tepperman *et al.,* 1970; Field and O'Dea, 1980; McCann and Hansel, 1986; Zubay *et al.,* 1995) for compensatory gain in weight (McDonald *et al*., 1998). The possible rebound response by the end of the second week was more significant ($P<0.05$) in the 5-6yr does, which gained 55.7g/day and 28.57g/day in HS and LS groups respectively, more than the corresponding gains in the 3-4yr does (Table 4.5; Fig 4.3).

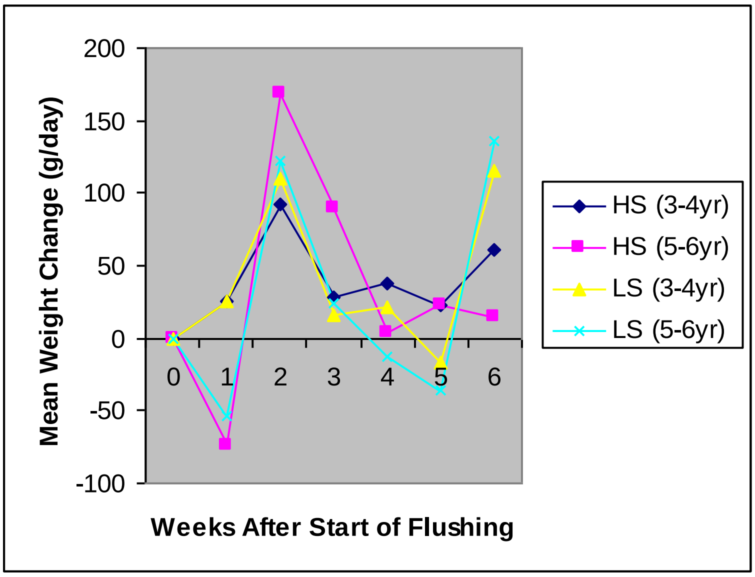
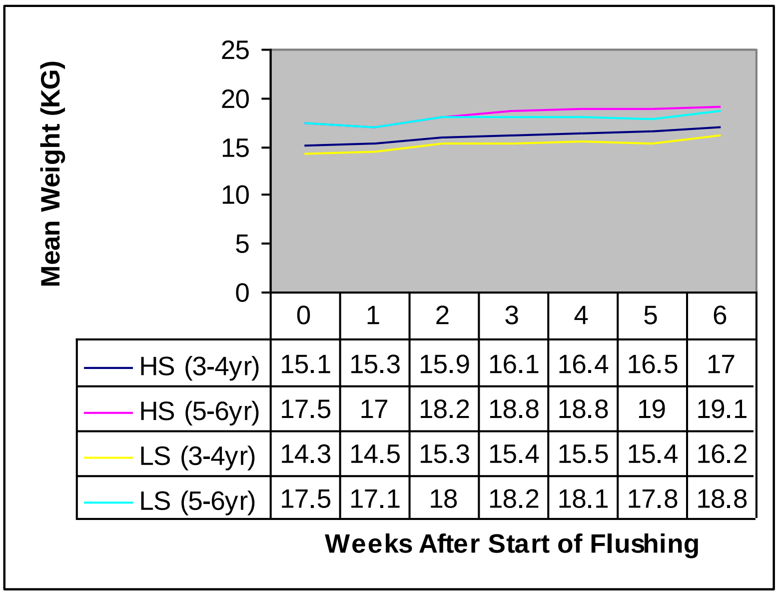


Fig 4.3 The Dynamic Effect of Flushing on Weight-changing Rate of Does According to Supplementation Level and Age



	0	1	2	3	4	5	6
HS (3-4yr)	15.1	15.3	15.9	16.1	16.4	16.5	17
HS (5-6yr)	17.5	17	18.2	18.8	18.8	19	19.1
LS (3-4yr)	14.3	14.5	15.3	15.4	15.5	15.4	16.2
LS (5-6yr)	17.5	17.1	18	18.2	18.1	17.8	18.8

Fig 4.4 The Weekly Apparent Growth Of Does According to Supplementation Level and Age

This might be because the 5-6yr animals, being relatively heavier (17.5kg), showed negative rate of weight gain (i.e. NEB) in the first week, perhaps, in continuity with a physiologic/bioenergetic need to reduce to the lower normal average weight of 16.5kg in line with the poor support of the dry season level of nutrition. In the process they must have been slower to recognize and adjust to the sudden improved plane of nutrition in the first week of flushing. Bioenergetically, such low NEB might have potentiated them for the greater rebound metabolic response to flushing in the second week.

By the third to fifth weeks, however, the entire experimental flock was declining to the lower weight and nutritional status prior to the start of flushing (Fig.4.3) perhaps, as a lipostatic effect (reduction in rate of weight gain towards a previous minimum body weight). According to Chesworth (1992) and McDonald *et al.* (1998) animals that live on low diets or low nutritional conditions over extended periods of time tend to adapt by reducing their feed intake and fasting heat production, preservation of relatively constant body weight and, therefore, a desire to return to that body weight if it is altered by starvation or forced feeding; and that this tendency is called the lipostatic theory or effect. Perhaps, the animals in this study became adapted to a low body weight or nutritional status prior to flushing and, therefore, tended to return to that weight level when suddenly exposed to higher plane of nutrition with both supplement and forage that had good %CP and energy density to meet estimated requirements for goats.

Thus, after compensatory gain in weight with rebound efficiency they became adjustably less efficient to maintain the high weight with the new nutritional status, and began to lose weight accordingly. It could be also that the animals recognized the high metabolic cost of maintaining the weight gained due to sudden improvement in plane of nutrition, and with memory of the immediate past dry season poor availability of feed (Archer *et al.*, 2002) tended to return to the more manageable lower and normal weight prior to flushing by imposing a self-regulation of feed intake as a measure against a probable food insecurity thereafter (Chesworth, 1992; McDonald *et al.*, 1998). It was during this period – after the second week – that the animals were observed to exhibit anorectic tendency by cutting down on grazing hours with a possible reduction in forage dry matter intake. It appeared that the anorectic tendencies were to some extent influenced by the administered luteolysin ($PGF_{2\alpha}$) on day 33 of flushing. The possible reduction in forage dry matter intake might also have been further enhanced by the reduced lush material from the preferred *Panicum maximum* that was ground-grazed selectively, while the other vegetation in the paddock rapidly overgrew and became poor in quality.

This apparent lipostatic response, however, appeared to have been modulated by the animals' adjustment to supplementation such that, although there were generally *diminishing rates of gain* in weight, only the LS groups that were discontinued with supplementation from the fourth week, had *negative (weight loss) rates* by the fifth week. At this time the LS group had returned to almost their initial average weight (15.9kg, Table 4.3) which was just below the Station average of 16.5kg at the end of the previous rains. The LS group were thus apparently primed for another rebound metabolism and responded accordingly to available quality forage during the last week of flushing by which time there was fresher lush material from re-growth of the earlier overgrazed *Panicum maximum* in the paddock, with resumption of the rains after a dip in the third week of flushing. The LS group's second rebound metabolism occurred in spite of discontinued supplementation, suggesting nutrient adequacy of the forage at this stage of growth and period of the season.

Meanwhile the HS group appeared to have adjusted to their continued supplementation with average weight of 17.7kg (by the fifth week) which was higher than the Station average of 16.5kg. With the higher level of feeding their body weight increased but with less efficiency and, therefore, at a lower rate than the rebound rate of the LS group (McDonald *et al.*, 1998), resulting in almost equal gains in weight for both the HS and LS groups at mating (Tables 4.3 and 4.4; Fig 4.3 and 4.4). The 10.25% body weight gain in the experimental flock falls slightly below the 12% observed for temperate sheep (Coop, 1969), but above the 6-8% suggested as practical for normal farm situations for sheep (Coop, 1969).

Table 4. 5 The Dynamic Effect of Supplementation Level and Age on Daily Body Weight Changes of Flushed Does

Treatment		Mean daily changes in weight (g)					
Supplement level	Age (year)	WK 1	WK 2	WK 3	WK 4	WK 5	WK 6
HS	3-4	54.28	100.00	87. 14	37.14	37.14	61.42
	5-6	-74.28	155.7	90.00	4.28	22.85	14.28
LS	3-4	18.57	94.28	18.57	27.14	-25.71	90.00
	5-6	-15.71	122.8	24.28	-12.85	-21.42	122.8

4.4.0 The Effects of Flushing on the Metabolic Profile of Does

Table 4.6 and Fig. 4.5 show the levels of the serum metabolites measured (mean±SD) at different stages during flushing. Differential tendencies due to main effects and treatment interactions were observed in the patterns of changing serum metabolite levels that resulted in the final metabolic profile at mating.

Table 4. 6 The Mean Concentration of Serum Metabolites at Three Different Stages during 45-Day Flushing Period According to Age and Supplementation Level of Does.

Treatment		Day 1			Day 33			Day 45		
Supplement Level	Age Year	Total Protein g/l	Glucose mM/l	Calcium mM/l	Total protein g/l	Glucose mM/l	Calcium mM/l	Total Protein g/l	Glucose mM/l	Calcium mM/l
HS	3-4	78.75±8.83	3.45±0.63	2.20±0.38	91.10±6.99	1.62±0.44	1.91±0.11	85.60±7.65	2.18±0.4	1.63±0.10
	5-6	81.05±10.81	2.95±0.07	1.89±0.10	92.07±7.35	1.56±0.43	1.79±0.12	87.54±4.03	2.38±0.75	1.53±0.09
LS	3-4	88.20±4.80	3.80±0.28	1.95±0.08	91.10±11.94	2.07±0.89	1.73±0.19	78.70±7.21	2.63±0.59	1.62±0.21
	5-6	81.65±2.61	3.30±0.00	1.69±0.11	92.00±10.20	1.53±0.45	1.71±0.10	83.17±9.72	2.67±0.46	1.64±0.22

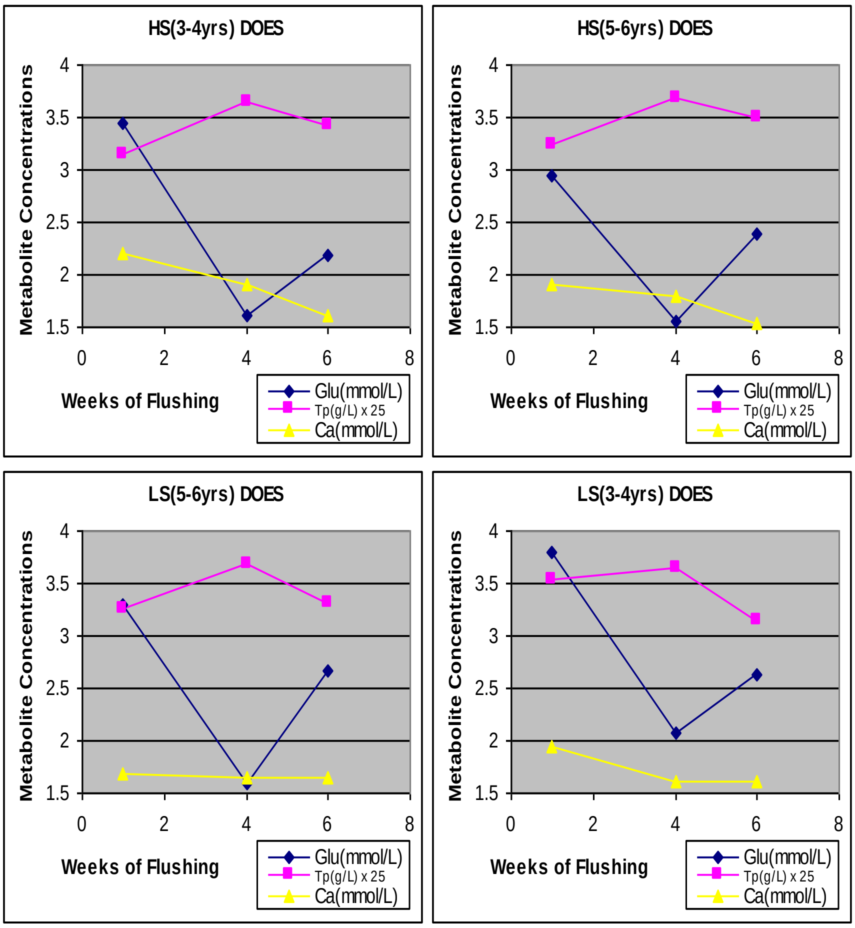


Fig 4.5 Effect of Supplementation Level and Age on Changing Serum Metabolite Concentrations of Does during Flushing

4.4.1 Total Protein

In Fig. 4.4 the mean serum total protein levels generally rose from day 1 to day 33 and then declined till end of flushing on day 45. However, there was a sharp rise and a gradual decline in HS than LS does; and in terms of age, both slopes tended to be more acute in the 3-4yr than 5-6yr does. Consequently, at mating, the main treatment effects indicated higher serum total protein concentration for the high than the low supplemented (86.57±6.03 v 80.90±8.64g/l) and for the 5-6yr than 3-4yr (85.32±7.61 v 82.15±8.05g/l) does, ($P<0.05$; Table 4.7) These general patterns of change were, however, modulated by effects of treatment interactions and were more pronounced in the LS (3-4yr) group. Thus at mating the serum total protein concentration of LS (3-4) group (78.70±7.21g/l) was significantly lower than for LS (5-6yr), HS (3-4yr) and HS

(5-6yr) groups (P<0.05), whose levels were 83.17±9.75g/l; 60±7.65g/l and 87.54±4.03g/l respectively (Table 4.8).

Table 4.7 The Main Treatment (Supplementation level and Age) Effects on The Metabolic Status of Flushed Does at Mating.

Parameters	Supplement level		Age	
	High supplemented does	Low supplemented does	3-4yr does	5-6yr Does
Mean serum total protein (g/l)	86.57±6.03^{a}	80.90±8.64^{b}	82.15±8.05^{b}	85.32±7.61^{a}
Mean serum glucose (mmol/l)	2.28±0.60^{a}	2.65±0.51^{b}	2.41±0.55^{a}	2.52±0.62^{b}
Mean serum calcium (mmol/l)	1.56±0.10^{a}	1.63±0.21^{b}	1.62±0.16^{b}	1.58±0.17b
Mean serum insulin (µIU/ ml)	15.09±7.39^{a}	19.06±13.59^{b}	19.82±13.78^{b}	14.32±6.44^{a}

Means (±SD) in the same row with different superscripts are significantly different (P<0. 05)

4.4.2 Glucose

Exactly the opposite pattern to that of mean serum total protein was observed in the changing levels of serum glucose (Fig 4.4). The general pattern was a fall in serum glucose concentrations from day 1 to day 33, and then a rise till end of flushing. In both high supplemented (HS) and the 3-4yr groups the fall was steeper and the rise gentler than in both low supplemented (LS) and 5-6 year groups of does. At mating main treatment effects showed that serum glucose concentration was higher in the low (2.65±0.51mmol/l) than high (2.28±0.60mmol/l) supplemented does, and for 5-6yr does (2.52±0.62mmol/l) than the 3-4yr olds (2.41±0.55mmol/l) (P<0.05; Table 4.7). Treatment interactions did not alter this pattern significantly though the LS (5-6yr) group tended to have a relatively higher glucose level (2. 67±0.46mmol/l).

4.4.3 Calcium

The mean concentration of serum calcium generally decreased consistently from the start to the end of flushing, with the decline being gradual in both HS and 3-4yr does than for the LS and 5-6yrr ones. However, at mating only the high supplemented (HS) does had serum concentration of calcium (1.56±0.01mmol/l) lower ($P<0.05$) than for LS, 3-4yr and 5-6yr does which had values of 1.63±0.21mmol/l, 1.62±0.16mmol/l and 1.58±0.17mmol/l, respectively. There was no significant difference in the calcium levels (mean±SD) at mating due to treatment interactions (Table 4.8).

Table 4. 8 The Interaction of Supplementation level and Age on Metabolic Profile of Flushed Does at Mating

Parameters	High supplement		Low supplement	
Metabolic Profile	3-4yr	5-6yrs	3-4yrs	5-6yrs
Mean serum total protein concentration (g/L)	85.60±7.65^{a}	87.54±4.03^{a}	78.70±7.21b	83.17±9.72^{a}
Mean serum glucose conc. (mmol/L)	2.18±0.43^{a}	2.38±0.75^{a}	2.63±0.59^{b}	2.67±0.46^{b}
Mean serum calcium conc. (mmol/L)	1.63±0.10	1.53±0.09	1.62±0.21	1.64±0.22
Mean serum insulin conc. (μIU/mL)	14.47±8.72^{a}	15.72±6.76^{a}	25.19±16.17^{b}	12.92±6.53^{a}

Means (±SD) in rows with different superscripts are significantly different ($P<0.05$)

4.4.4 Insulin

Generally, low level of supplementation and 3-4yr olds (19.06±13.59μIU/ml and 19.82±13.78μIU/ml, respectively) favored higher serum levels of insulin at mating ($P<0.05$) than high supplementation and 5-6yr olds (15.09±7.39μIU/ml and 14.32±6.44μIU/ml). Thus for treatment interactions significant difference ($P<0.05$) was noted for only LS(3-4yr) group, which had mean level of 25.19±16.17μIU/ml compared to 12.92±6.53, 14.47±8.72 and 15.72±6.76μIU/ml for the LS(5-6), HS (3-4) and HS (5-6yr) groups, respectively.

The pattern of change in the levels of serum metabolites during the first four weeks of flushing (Fig. 4.5) may be associated with the effects of earlier lactationally induced metabolic status of the does and rebound metabolism in response to supplementation. Since the start of the experiment coincided with the end of 3-4 months of lactation and scarcity of feed serum glucose and calcium levels were, perhaps, falling within physiological range due to reduction in lactation demand and the consequential demobilization from tissue reserves. Zubay *et al.,* (1995) explain that to meet the fluctuating demands for energy and intermediary metabolites living organisms adjust the rates of metabolism over broad physiological ranges so as to attain a steady state between the synthesis and utilization of the metabolite for a particular biological process; and that in vertebrates when dietary glucose level in circulation falls, the liver mobilizes glucose from reserves in tissues to maintain normo-glycemia. Similarly, the total protein rising in serum levels may be due to reduced absorption of some fractions of the blood protein by mammary glands with the ending of lactation. McDonald *et al.,* (1998) stated that a fraction of milk protein made up of small amounts of α-lactalbumin, serum albumin and the immune globulins, pseudo-globulin and euglobulin are all absorbed directly from the blood. In addition, the changing metabolite pattern may suggest also a rebound increase in the uptake and utilization of both circulating glucose and amino acids for increased protein synthesis due to the sudden increase in both dietary availability of these metabolites and sensitivity to insulin action (Tepperman *et al.,* 1970; Field and O'Dea, 1980; Zubay *et al*., 1995).

That the rise in total protein and the fall in both glucose and calcium were sharper for HS and 3-4 year does suggests that higher protein synthesis and calcium assimilation rates were favored by high supplementation and 3-4yr age. This may also be due to the higher total dry matter intake of supplement by the HS and the greater apparent compensatory growth rate of the 3-4 year does. An animal which receives higher level of nutrition gives a high level of production whether it is milk, meat or physical work (Chesworth, 1992). As alluded to earlier, the possible rebound metabolism effect seemed reversed by the apparent lipostatic responses of the animals to the improving body condition from the end of the second week and became more obvious by the end of the fourth week (day 28). This may explain the decline in physical condition, hyperglycemia and hypoproteinemia observed by the beginning of the fifth week (day 33) to end of flushing. Furthermore, the apparent lipostatic condition (tendency to maintain previous weight or metabolic status) of the animals appeared to have been deepened by sustained anorexigenic effects (reduction in appetite, food-intake and weight), perhaps, due to estrual synchronization with Estrumate (Cloprostenol) – a prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) analogue. Prostaglandins (PGs) are rapidly degraded in the blood, but injection with pharmacologic or high levels can result in sustained physiologic effects (Hafez, 1992) such as ischemia or lack of blood supply to tissues (Frandson, 1974), hyperglycemia (Oliveira *et al.*, 2003;Flores *et al.*, 2004; Monnier *et al.*, 2006)) and concurrent hyperleptinemia cum hyperglycemia (Georgescu and Popou, 2003). Perhaps for the light WAD does (average live weight = 16 kg at the time of PG administration) dosage of 62.5 micro grams per doe was just physiologically intolerable, resulting in sustained physiologic problems with blood circulation and glucose metabolism. Thus the four does in this current study which exhibited anorectic tendencies of reduced food intake and weight loss till death (7 to 8 weeks) postpartum may have had their lipostatic condition worsened by the $PGF_{2\alpha}$ injection. Owiredu (personal communication)[5] concluded that a possible explanation may be that the excess level of $PGF_{2\alpha}$ antagonized insulin’s metabolic function (or induced insulin resistance in body cells) leading to low uptake of glucose and amino acids for protein synthesis and resulted in the hyperglycemia, hypoproteinemia and gradual decline in weight. Fonseca *et al.,* (2004) administered 22.5 µg/doe cloprostenol to Toggenburg does with 41kg average live weight and did not report any observed sustained physiologic effects.

Meanwhile, by the end of the fifth week it appears a second rebound metabolism was initiated. The LS group having had deeper loss of energy in the apparent lipostatic phase responded more readily than the other groups to

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this second rebound metabolism. This was further evidenced by the significantly higher serum level of insulin (25.19±16.17µIU/mL, $P<0.05$) in the LS (3-4yr) group that had the lowest energy balance or weight loss (-25.71g/day) during the lipostatic phase. According to McCann and Hansel (1986) re-fed heifers that had been restricted feed for 8 days responded with rebound metabolism and with concomitant increase in plasma concentration of insulin and glucose than the fed controls

These dynamic effects of flushing on serum metabolites and insulin resulted in the metabolic profile at mating, that is, day 45 of flushing (Table 4.8) The mean±SD serum levels of total protein at mating was statistically the same in all the treatment groups except for LS(3-4yr), which had the lowest ($P<0.05$) level (78.70±7.21g/L). However, even this lowest level was higher than a range of 58.4±0.42 to 61.3±0.52g/l reported for 1-2 year WAD bucks supplemented in Nigeria with leguminous browse for 8 weeks (Ajala *et al.,* 2000). The difference in their result and the current study may be attributed to the lower age and sex of their animals. Tighe and Brown (2003) provide reference interval of 59.0 to 74.0 g/l for Canadian goats and explain that the levels vary from laboratory to laboratory and depend on the method used, as well as the breed, sex, age and environment of the animal. The range for the WAD does in the current study was 59.2 – 98.2 g/l, which extends to higher limits than for the Canadian goats and 60 – 80g/l for temperate ovine species (Blood *et al.,* 1983), but lower than the mean of 102.6g/l (10.26mg/100ml) for non-pregnant Djallonke gimmers in Ghana (Obese *et al*., 1994). Perhaps, this higher values of the WAD does (and sheep, Obese *et a*l., 1994) reflect a relatively more dehydrated status due to tropical (semi-arid) climate or rising metabolism at the time due to improvement in pasture with the rains, since total protein levels can be altered by the level of dehydration or over hydration (Tighe and Brown, 2003).

At mating only LS (3-4yr) group had mean serum total protein concentration that was significantly lower (P<0.05) than for the others. Generally, there was clear tendency of an overall increase in protein synthesis due to high supplementation though the rate of replacing serum total protein tended to be slower in 3-4yr than 5-6yr does. This may imply that 3-4yr does have had a more severe lactation protein distress than older does prior to the experiment (Mtimuni, 1995) which, coupled with the rather low supplementation, resulted in the significantly lowest value for the LS (3-4yr) group.

Similarly, the serum glucose uptake/or utilization was significantly improved (P<0.05) by both high supplementation and 3-4yr age with the greatest uptake in the HS (3-4yr). This was at variance with Tanaka *et al. (*2004) who evaluated the effects of dietary restriction on metabolic, ovarian and endocrine profiles of goats prebreeding and reported no significant difference in weekly plasma glucose levels for both treated and control animals. The range of 1.4 to 4.0mM/l is lower than the 3.2 to 4.2 for Canadian goats (Tighe and Brown, 2003), perhaps because of climatic differences. For instance in an investigation to evaluate the physiological response of female yaks under different environments (Sarkar *et al.*, 2000) found that their blood glucose levels were higher in cold humid (winter) season than in the moderately cold (summer) season. According to Bergman (1963) the normal blood glucose level for clinically healthy ruminants range between 45 –65mg/100ml (2.49-3.61mM/l), and Obese *et al*. (1994) had a mean of 3.05mmol/L (55.97mg/100ml) for non-pregnant gimmers in Ghana.

Calcium assimilation rates were not significantly different (P>.05) except for the HS (3-4yr) group. The graphical presentation suggests a relatively faster removal from serum due to high supplementation and young age, concomitant with the general influence of these two factors on the overall metabolism of the flushed does. The non- significant influence of treatments on the calcium values at mating may suggest the range 1.36 to 2.03 mmol/l as a basal physiological serum level for the 3 - 6 year WAD does, in their follicular phase of estrous cycle. This range, however, is lower than the 2.12 – 2.55mmol/l for Canadian goats (Tighe and Brown, 2003) and the mean of 2.47mmol/l (9.89mg/100ml) determined for non-pregnant Djallonke gimmers in hot humid ecozone in Ghana (Obese *et al.,* 1994). Perhaps the smaller skeletal frame size of WAD breed goats implies lower calcium metabolism since 99% of body calcium is in bone (Tighe and Brown, 2003).

Generally, neither high supplementation nor 5-6yr age seemed to have influenced the mean serum concentration of insulin at mating ($P>0.05$) as did low supplementation and 3-4yr age. Thus with treatment interactions mean serum insulin concentration at mating was significantly higher ($P<0.05$) in LS (3-4yr) than for the other treatment groups. However, except for group LS (5-6yr), which had the lowest and yet highest values of insulin and glucose respectively (Table 4.8), there was a weak positive correlation ($r = +0.0731$) between insulin and glucose levels at mating in the entire experimental flock. Probably, glucose levels just before mating induced the increase in serum concentration of insulin. Circulating insulin level can be influenced by recent energy intake (Havel, 2001) and circulating protein, which act as a potent stimulator of insulin secretion (Peters and Mayer, 1993). The significantly higher levels observed in the LS(3-4yr) group of does may be the result of their ability to change more quickly than the others from lipostatic conditions in the fifth week to rebound metabolism in the last (6th) week of flushing in response to improvement in pasture (McCann and Hansel, 1986). This agrees with the observation that plasma concentration of insulin in fasted heifers returned from fasted level of 162±5pg/ml to control values of 773±86pg/ml 12h after refeeding and continued to increase more than the controls ($P<0.05$) in a linear fashion for the first 6 days of refeeding (McCann and Hansel, 1986). The relative hypoproteinemia of the LS (3-4yr) does at mating suggests that their relative hyperinsulinemia may be due to increased secretory than synthetic activity of their pancreas. The range for mean insulin at mating in this study was 6.8-44.04mIU/ml.

4. 5. 0 Effect of Flushing on Pituitary Functioning Status of Does at Mating

The serum concentrations of LH and FSH measured at 6h intervals during follicular phase for 24h to index effect of flushing on functioning status of the anterior pituitary at mating are shown in Tables 4.9 and 4.10.

Table 4.9 Main Treatment (Supplementation Level and Age) Effects on The Pituitary Functioning Status of Flushed Does.

Main Treatments	Supplement level		Age	
Parameters	High supplemented does	Low supplemented does	3-4yr does	5-6yr Does
Mean serum FSH (mIU/ml)	0.295 (n=1)	18.9 (n=1)	18.9 (n=1)	0.295 (n=1)
Mean serum LH (mIU/ml)	6.54±13.00^{a} (n=10)	7.13±15.16^{ac} (n=10)	11.84±17.87^{c} (n=10)	1.83±4.92^{b} (n=10)

Means (±SD) in the same row with different superscripts are significantly different ($P<0.05$)

N = number of observations

Table 4.10 The interaction of Supplementation Level and Age on Pituitary Functioning Status of Flushed Does at Mating

Parameters	High supplement		Low supplement	
	3-4yr	5-6yrs	3-4yrs	5-6yrs
Mean serum FSH conc. (mIU/mL)	<DL	0.39	18.99	<DL
Mean serum LH conc. (mIU/mL)	9.63±17.58^{a} (n=10)	3.45±6.91^{a} (n=10)	14.05±19.93^{a} (n=10)	0.21±0.36^{b} (n=10)

< DL = lower than detection limit. N=number of observations. The figures for FSH were for n=1

4. 5.1 Luteinizing Hormone (LH)

The mean serum LH concentration of the low supplemented does (7.13±15.16mIU/ml), though apparently higher, was not different (P>0.05) from that of the high supplemented (6.54±13.00mIU/ml); but higher values favored 3-4yr more (P<0.05) than 5-6yr age (11.84±17.87mIU/ml v 1.83±4.92mIU/ml, respectively; (Table 4.9). Consequently, the treatment interactions on LH concentrations at mating were higher in LS (3-4yr) does (14.05±19.93mIU/ml) but not significantly different (P>0.05) from the others except for LS (5-6yr) does with a value of 0.21±0.36mIU/ml (Table 4.10). The difference between serum LH values for the 3-4yr and 5-6yr does (Table 4.9) may be because the 3-4yr animals had a significantly higher glucose uptake (Table 4.7; Ohkura *et al.,* 2004), which may suggest a positive correlation between serum LH and glucose metabolism. Contrarily, between the two 3-4yr groups, the LS (3-4yr), [which had overall higher mean serum glucose concentration - implying lower peripheral glucose uptake – than the HS (3-4yr) (2.63 v 2.18 mmol/l, respectively)], had higher LH (14.049 v 9.630 mIU/ ml) at mating. This may be due to the rebound metabolism of LS (3-4) group in the last week (McCann and Hansel, 1986). Moreover, reproductive neuroendocrine output (GnRH/LH) is stimulated by current increased food intake and not by high adiposity (or body reserve) in sheep (Archer *et al.,* 2002). Perhaps, the rebound metabolic response influenced increased secretory rather than biosynthetic activity of the pituitary since this group showed also relatively lower proteinemia during the follicular phase at mating.

No reference levels of LH in goats *per se* were obtained for comparison in the reviewed available literature of the current study. However, in sheep basal level of LH in peripheral plasma rarely exceeds 2-3ng/ml and the preovulatory peak at estrus (±8h) rises to 20-100ng/ml and lasts for 6-8h (Hunter, 1980; Gatenby, 1986). Holstein plasma levels of LH were found to range from 36.0 ± 7.29 ng/ ml (mean±SE) on hour 0 (i.e. beginning) of estrus to 1.5 ± 0.08 ng / ml on hour 30 (ovulation time) in the dry season; the corresponding figures for the rainy season were 12.1± 3.84ng/mL and 1.6±0.37ng/nL (Morales *et al.,* 1990).

Morales *et al.* (1990) reported that maximal plasma LH levels were found 0-6h after the first manifestation of estrus, returning to base values 9-12h later; and that seasonal variations did not influence the results though the preovulatory peak was more marked in the dry than the rainy season. This suggests that irrespective of season, peak or highest LH values are detectable around the time of estrus. Hence, the values in this study may be not only within the physiological range but also near the peak values for goats.

4.5.2 Follicle Stimulating Hormone (FSH)

Only does # 0472 and # 0375 belonging to the HS (5-6yr) and LS (3-4yr) treatment groups, respectively, had values (0.295±0.297 and 18.99±1.46 mIU /ml) for FSH detected from blood samples taken between 31 and 69 h (inclusive) after the PGF_{2a} second injection. No detectable values could be obtained from similar blood samples of all the other 18 experimental animals selected randomly for their gonadotropin assay. Between the two does with detected values only doe # 0375 showed behavioral estrus; and it was at 71h after PGF_{2a} second injection. This suggests that its estrual or preovulatory FSH surge started earlier and was detectable 31h and beyond with, perhaps, pulsatility (measured 6-hourly) reaching peak at 49h after second PGF_{2a} injection (that is to say 22h before estrus, Fig 4.6).

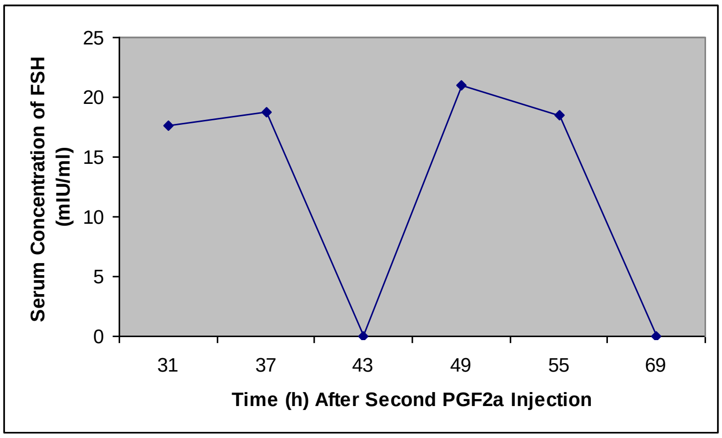

Fig 4.6 Serum Concentration of FSH hours after $PGF_{2\alpha}$ Second Injection in Doe # 0375

During the follicular phase of estrous cycle in goats there is a preovulatory LH and FSH surge, with a peak that lasts from 6-12hours (Hafez, 1993).

The absence of detectable values of FSH may be due to causes such as inaccurate prediction of fixed sampling time relative to estimated time for incidence of synchronized estrus or/and insensitivity of the human serum kits to concentrations of similar molecules in goats. From Table 4.11, more than 50% of the experimental does exposed to the buck showed behavioral estrus before the 60th h after PGF_{2a} second injection, suggesting that the pre-estrual/ovulatory FSH surge for 50% of the experimental flock must have occurred even much earlier than the first blood sampling at 31h post PGF_{2a} injection. On the other hand, even **after** the last sampling (69th hour post PGF_{2a} injection), some of the total of about 33.3% of the flock left in the mating pen came on heat. Therefore, the undetectable values for FSH may be due to difficulty in predicting blood sampling time in relation with estrus synchronization. Perhaps it might also be because of insensitivity in goat serum of the human kits, which had detection limits of either 0.8 IU/L (Human Co) or 2.1mIU/mL (Calbiotech Ltd). It appears that some human-hormone-kits may not be directly suitable for animal blood assays unless diluted with given concentration factors (Tighe and Brown, 2003). This is, however, doubtful in this study considering that the Calbiotech kit detected values as small as 0.142mIU/ml with its detection limit of 2.1mIU/ml.

4. 6. 0 Effects of flushing on estrus synchronization

The time interval between second PGF_{2a} injection and estrus and its distribution according to supplementation level and age are shown in Table 4.11 On the whole there were no significant differences among the mean ± SD for time interval to estrus due to treatment, thus allowing pooling of the experimental flock mean ± SD interval to 60.17 ± 20.20 h, with 87.09% does on heat within 126h.

There were significant individual variations, however, in the time of estrus between supplementation treatment groups, the LS group of does tending to come on heat earlier with at least 70% within 32 - 59h after 2nd $PGF_{2\alpha}$ injection as against 20% - 40% of the HS – with the latter having 30% anestrous does.

Table 4.11 Time Interval Between 2nd $PGF_{2\alpha}$ Injection and Estrus and its Distribution in Flushed Does According to Supplementation Level and Age.

Supplement level	Age (yr)	Time(h) interval to estrus (mean ± sd)	Distribution of time interval to estrus (%does exposed to the buck)				
			Early Estrus (32-59h)	Mid Estrus (60-72h)	Late Estrus (78-80h)	V. late Estrus (100-126h)	Anestrus (>126h)
HS	3-4	56.28±19.85	40.0	10.0	20.0	0.0	30.0
	5-6	72.62±27.52	20.0	20.0	30.0	10.0	20.0
LS	3-4	57.70±18.83	70.0	20.0	0.0	10.0	0.0
	5-6	54.88±13.28	77.7	11.1	11.1	0.0	0.0
Overall	3-6	60.17±20.20	51.28	15.38	15.38	5.13	12.80

Between the age groups the 3-4yr does tended to have a close time interval between HS(3-4yr) 56.28±19.85h and LS(3-4yr) 57.70±18.83h; the 5-6yr groups on the other hand were apart with the HS(5-6yr) time interval of 72.62±27.52h being almost significantly higher than that of the LS(5-6yr) counterpart (54.88±13.28h) (t_{cal} = 2.105; LSD=2.365). It was not certain whether higher supplementation could be implicated, but overfeeding prepubertal goats decreases fertility and impair mammary gland development (Wildeus, 1996). Moreover, although synchronization (luteolytic or progestagen) treatment reduces variations in timing of estrus compared to a natural estrous cycle there is still a significant individual variability inherent in delays following estradiol peak, which are beyond capacity of synchronization (Freitas *et al.*, 2004). This individual variability is partially explained by the dynamics of the dominant follicle (DF) at the time of induced luteolysis. If regression of the corpus luteum (CL) occurs when a DF is in the rapidly growing stage then ovulation may occur in 2-3 days. If on the other hand the DF is static or in fact had already begun to regress when luteolysis is induced, then a longer period of time is required for a new DF to emerge, grow and ovulate-3-5 days (O S U, 2001). Perhaps, the experimental flock had appreciable individual variation in the DF maturation even at the time of the second luteolytic treatment, resulting in the wide response time range of 32 to 126h.The percentage of does in estrus (87.09%) is lower than the 93.3% and the mean interval from PGF_{2a} second injection to estrus (60.17±20.20h) is higher than the 41.1±9.3h reported by (Fonseca *et al.*, 2004) for Toggenburg does treated with 6 days CIDR (controlled internal drug release) progestagen and 22.5µg cloprostenol/doe. Since these authors also detected estrus using bucks, the progestagen – prostaglandin combination, perhaps, helps to reduce individual variability in the timing of estrus in synchronized animals (Freitas *et al.*, 2004) than prostaglandin alone.

4. 7. 0 Effect of flushing on reproductive performance of does

The fertility rate (calculated as percentage of does that successfully conceived of does exposed to the buck), prolificacy (kid per conception) and other reproductive parameters are shown in Tables 4.12 and 4.13

Table 4.12 Main Treatment (Supplement Level and Age) Effects on The Reproductive Performance of Flushed Does.

Main Treatments	Supplement level		Age	
Parameters	High supplemented does	Low supplemented does	Younger (3-4yrs) does	Older (5-6yrs) Does
Fertility rate (%)	75.00	73.68	75.00	73.68
Prolificacy (kid/conception)	1.80	1.50	1.80	1.50
Twinning rate (%)	66.66	50.00	66.66	50.00
Triplet rate (%)	6.66	0.00	6.66	0.00

Table 4.13 Interaction of Supplementation Level and Age on the Reproductive Performance of Flushed Does

Treatment		Reproductive performance			
Suppl. Level	Age (yr)	Fertility (%)	Twinning (%)	Triplet (%)	Prolificacy (kid/conception)
HS	3-4	70.0	85.7	14.30	2.14^a
	5-6	80.0	50.0	0.00	1.50^b
LS	3-4	80.0	50.0	0.00	1.50^b
	5-6	66.6	50.0	0.00	1.50^b

Means in a column with different superscripts are significantly diffrernt. (P<0.05)

4.7.1 Fertility Rate

On the average neither supplementation level nor age affected the fertility of does in this experiment although there was tendency of higher fertility with both high supplementation and 3-4yr age (75.00%) than low supplementation and 5-6yr age (73.68%). The overall average for the entire experimental flock was 74.3% after first service (Table 4.12). Fertility in does declines with age (Peacock, 1996; Kinne, 2000). Between the two 3-4yr groups, LS (3-4yr) were more fertile (80%) than the HS (3-4yr) (70%; Table 4.13). This could result from the higher serum insulin and LH concentrations of the former group at mating. Both insulin and LH interact synergistically at the ovarian level to improve steroidogenesis of estradiol and ovulation (Webb *et al.*, 2004), which are necessary conditions for good fertility (Mukasa – Mugerwa, 1989). The overall fertility of 74.3% falls within the annual rate of 71.4 to 160[6] percent in Sub-Saharan Africa (SSA) (Abassa, 1995) but lower than the 83.3% annual rate over 5 years reported for the mother flock from which was selected the experimental animals (40) (Baffour – Awuah *et al.*, 2004). The discrepancy

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[6] Abassa (1995) defined fertility as number of parturitions per female of breeding age per annum; hence the possibility of obtaining values >100% since a female can kid more than once in a year.

may be due to the differences in numbers (886 v 39) and duration of reproductive activity (5 yrs v 5 months; Abassa, 1995).

4.7.2 Kidding Performance

Analysis of variance indicated significant differences (P< 0.05) in treatment interactions on prolificacy. Table 4.12 shows that both high supplementation and 3-4yr age favored higher prolificacy (1.80kids/conception) than low supplementation and 5-6yr age (1.50kids/conception). However, the interaction of supplementation level and age was observable more in the HS (3-4yr) group, with higher twinning and triplet rates of 85.7% and 14.30%, respectively than the rest of the groups, bringing its prolificacy to 2.14 (or 214%)(Table 4.13). Since only one group responded positively to treatment the pooled results were 58.6%, 3.5% and 165% for twinning, triplet and prolificacy rates, respectively in the entire experimental flock (Table 4.13).

Although low supplemented and 3-4yr does had higher pituitary and pancreas functioning at mating it was the combination of high supplementation and 3-4yr age that showed higher prolificacy as demonstrated in the respective values of these parameters for LS (3-4yr) and HS (3-4yr) does. Increased circulating levels of insulin (Harrison and Randel, 1986) and LH (Lucy, 2003) are believed to be associated with increasing ovarian activity, ovulation rate and hence higher probability for multiple birth. Therefore, the higher multiple birth rates of HS (3-4yr) than for LS (3-4yr) suggest that endocrinological advantage at mating alone may not be sufficient to ensure improvement in multiple births. Probably the physical / metabolic status, such as the higher gain in body condition of the HS (3-4) may be equally necessary, together with the increased endocrine activity. Multiple birth is associated with female animals that have gained from a poor to moderately good condition (Nix, 2004) and it is inferable from Meza-Herrera *et al.* (2004) that the benefits of flushing are more obtainable when it leads to both rapid metabolism – of hormones of the energy/reproductive axis - (the dynamic physiological effect) and improved metabolic status (static physical effect). Perhaps the does in LS (3-4) group had the dynamic (endocrinological) but inadequate static (physical / metabolic) improvement. Improvement in physical/metabolic status pre-breeding may be needed to provide energy for reducing possible pre-embryo losses – especially failure of ovulation or/and abnormally small and incompetent ova (Fogwell, 1997), failure of ovum pick up (Hafez, 1993) and fertilization failure (Mapletoft *et al*., 1986). Thus, with the higher levels of serum insulin and LH at mating, the does in LS (3-4yr) group appear to have increased ovarian activity, but also higher pre-embryo losses. It may also be that a higher number of individuals failed multiple ovulations or multiple fertilizations and, subsequently, lowered chances for high prolificacy (McKenzie and Terril, 1937).

The prolificacy of the entire experimental flock of 1.65kid / conception is within the range of 1.05 to 1.87 kid / doe described for SSA and close to the highest value (1.87kid/doe) reported for Ghana (Abassa, 1995). It is higher than the 1.27kid/doe calculated over five years for the mother flock of the experimental does (Baffour-Awuah *et al.,* 2004), suggesting a positive benefit of flushing, despite any differences due to flock size and length of breeding period. Similarly the twinning rate (58.6%) is higher than the 50.2% (Baffour – Awuah, 2004), and compares very well with the range 13.5 to 60.4% reported for SSA, of which the highest from Ghana's humid ecozone was 59.0% (Abassa, 1995). It is also higher than the 43.7% for goats in the semi – arid ecozone of Ghana (Karbo *et al.,* 2003). In the same way triplet rate of 3.5% is far higher than 1.6% for the mother flock (Baffour – Awuah *et al.,* 2004) perhaps due to both flushing and the comparatively smaller number of the experimental flock.

4.8.0 Relationships among the Metabolic, Physical, Endocrinologic and Reproductive Indices due to Flushing.

A summary of the parameters indicating the physical, metabolic, and endocrinological status of the does at mating after flushing, as well as their reproductive performance at parturition have been presented in Tables 4.3 to 4.13 and figures 4.1 to 4.5. The metabolic profile at mating consists of serum concentrations of three metabolites and a metabolic hormone (insulin) while the physical status is characterized by body measurements of weight and condition score, including absolute and relative body weight gains. Serum concentrations of LH and FSH indexed the pituitary activity at mating while the reproductive performance considered fertility, prolificacy and multiple birth rates. The correlation coefficients for interrelationship among these parameters according to level of supplement and age are presented in Tables 4.14 to 4.16 and figure 4.6.

4.8.1 Relation between Insulin, Serum Metabolites and Body Mass Gains

Physiologically both the physical and reproductive benefits of flushing are believed to result from general rapid metabolic rate (Belows *et al.,* 1973; Cox *et al.,* 1987) with increasing levels of circulating dietary and intermediary metabolites (Zubay *et al.,* 1995). Moreover metabolism is intracellular and nutritionally induced circulating insulin is known to regulate the cellular uptake of circulating glucose and amino acids (Etherton, 1982; Zubay *et al.,* 1995). In addition, circulating insulin and leptin concentrations are proportional to body fat content; however the secretion and circulating levels are also influenced by recent energy intake and dietary micronutrient content (Havel, 2001). Presumably correlations may be expected between glucose, total protein, body mass gain and insulin at mating. Table 4.14 shows the correlation coefficients for the interrelations between serum insulin levels at mating and the metabolic and physical parameters of the experimental does. Generally they are low correlations except -0.8571 and -0.7024 which are high, but not significant.

Table 4. 1 4 Correlation Coefficients (r) For Interrelation between Serum Insulin Levels at Mating and Metabolic/Physical Parameters of Flushed Does According to Supplement Level and Age.

Parameters (Correlation between mean insulin levels and…)	High supplement		Low supplement	
	3-4yrs (n)	5-6yrs (n)	3-4yrs(n)	5-6yrs(n)
Serum glucose concentration (r)	- 0.3768	+0.1944	-0.1568	+0.3550
Serum total protein Concentration (r)	+0.2073	+0.5819	+0.3440	-0.5164
Gain in BCS (r)	-0.3564	-0.5048	+0.5144	+0.3500
Gain in body weight (r)	+0.2893	-0.8571	+0.2076	-0.7024

(n = Number of observations =5)

4. 8.1-1 Insulin and Body Weight

Age influenced correlation between serum insulin concentration and weight gain in does at mating, the relation being stronger and negative in older does (r = -0.8571 and -0.7024 for HS and LS groups respectively, which fall just below significance: $r_{critical} = 0.8780$, P = 0.05). The strong negative correlation in the 5-6yr does may suggest some effect of aging on sensitivity to insulin action such that reduction in weight is associated with increased insulin levels, because age may affect blood macromolecules (Tighe and Brown, 2003). Further more, the apparent negative correlation may be a tendency for lipostatic response to any initiated gains of body mass with, perhaps, increased forage intake and hence increased insulin levels in the last week (McDonald *et al.,* 1998; Havel, 2001). The 5-6yr goats tended to maintain constant body weight and so were the first to show apparent lipostatic responses to flushing, perhaps based on their nutritional memory (Archer *et al*., 2002) and their pre-flushing average weight of 17.5kg that had exceeded the 16.5kg and fell just below the 18kg mean and maximum weights, respectively at the Station.. There was no significant but positive relation between insulin and body weight change in the 3-4yr does which may be expected from insulin's metabolic influence on growth (Zubay *et al.,* 1995). One - and - half year (19-month) does supplemented with by-pass protein depicted higher serum insulin levels (Meza - Herrera *et al.*, 2004).

4. 8.1- 2 Insulin and BCS

Insulin's relation with body condition (adiposity) was insignificant (P>0.05) but was directionally affected by level of supplementation, the high and low supplement does being negative and positive, respectively. This deviates from the report of Meza - Herrera *et al*. (2004) that high BC does had the larger serum insulin concentration than the low ones, but did not state if there was a correlation. Perhaps the animals in this study were accustomed to poor nutritional status, resulting in less alteration in nutritionally induced insulin-influenced lipogenesis; and therefore, suggesting a need for more time to adjust both mentally and physiologically to the new levels of feeding. Animals have the tendency and desire to return to a previous constant body weight kept over time if that weight is altered by any forced feeding (McDonald *et al.,* 1998), because of nutritional memory (Archer *et al*., 2002) and as adaptation for poor nutritional times and environment (McDonald *et al.*, 1998). Thus, while the HS group tended to reduce any further weight increase the LS group tended to upgrade any losses in weight through the action of nutritionally induced circulating insulin. A closer study of the absolute value of the correlation coefficients reveal that with 3-4yr does the relation between insulin and BCS may favor tendency to improve BCS in poor condition does than to reduce it in good nutritional situations. It is the other way round (high tendency to reduce condition of well fed than improve condition of poor fed animals) in the 5-6yr does. It appears that the long (5months) dry season at Kintampo with characteristic poor availability of feed significantly imprint a nutritional memory and physiology that may require, at least, 6 weeks of adjustment supplementations to improve BCS and serum insulin levels before breeding; and that the 3-4yr does are more likely to respond positively to such adjustment supplementation regime than 5-6yr does.

4.8. 1-3 Insulin and Serum Metabolites at Mating

The correlations between mean serum insulin, glucose and total protein at mating were not significantly (P>0.05) affected by age or supplementation, contrary to Meza - Herrera *et al.'s* (2004) report that by-protein supplementation increased serum insulin. Nevertheless, the direction of the correlation with glucose in this study was influenced by age being negative in the 3-4yr does and positive in the 5-6yr does. This suggests that with advancement from 3-4yr to old 5-6yr the relation between insulin and glucose at mating may follow an inverse pattern such that serum glucose level falls within physiological limits (implying high utilization) with increasing insulin in 3-4yr does, but in older does, both either increase or fall proportionately together with change in diet. Moreover, between the two 5-6yr groups those with low supplementation showed higher positive correlation, suggesting a greater fall in insulin level in case of energy stress. This agrees with Whitaker *et al.* (1993) who stipulate that animals in negative energy balance are characterized by hypoinsulinemia. They did not refer to any age differences, but perhaps reported on older animals.

In the case of total protein the correlation with insulin was generally positive, except for does in LS (5-6yr) group, suggesting increasing serum level of insulin associated with increasing serum level of total protein at mating. Perhaps, the HS (5-6yr) group also had the negative correlation, in concomitance with the general observation of body mass loss with increasing insulin levels in the 5-6yr does (Table 4.1.4), but for a strong modulation by the concentrate supplementation to positive correlation.

These results show that at mating or follicular phase, serum insulin may not only increase but may be associated with reducing levels of serum glucose and rising levels of serum total protein, especially in the 3-4yr does. Possibly, there was increased sensitivity to insulin influenced uptake of circulating dietary glucose and amino acids (Etherton, 1982) due to rebound metabolism upon supplementation or improvement in forage (McCann and Hansel, 1986). It may in turn lead to increased anabolism and or secretion of biological (including reproductive) macromolecules (Zubay *et al.,* 1995). Increased plasma insulin was associated with increased plasma LH (McCann and Hansel, 1986). Impliedly, the effects of flushing on reproductive parameters such as behavioral estrus, ovulation rate and hence, high prolificacy probability may be more pronounced in younger than 5-6yr does, since the former seem to have more favorable correlation between insulin and serum metabolites from reproductive point of view. Nutritionally induced changes in serum insulin level are strongly related to improvement in the IGF-system, which enhances follicular steroidogenesis (Spicer and Echternkamp 1993; Williams *et al.,* 1997) – a potentiation of high fertility (Hafez, 1992). With insulin-induced increase in LH and ovarian activity high reproductive response should be expected (Lucy, 2003). This conclusion has evidence from the current study in which both observatory and arithmetic calculations indicate higher early behavioral estrus (55% v 48.85%) fertility (75% v 73.6%) and prolificacy (1.80 v 1.50 kids / conception) in the 3-4yr (especially the high supplemented) than the 5-6yr does.

4.8.2 Relation between Mean Body Mass and Mean Serum Total Protein at Mating.

Both body condition score (BCS) and body weight at mating tended to correlate with serum total protein negatively in the 3-4yr but positively in the 5-6yr does (Table 4.15). The correlation coefficients for BCS were -0.5346 and -0.0661 for younger and +0.4199 and +0.4548 for the mature does in the HS and LS groups respectively.

Table 4.15 Correlation Coefficient for Relaton between Mean Serum Total Protein and Body Mass Change at Mating According To Supplemention Level and Age

Parameters Correlation between mean	High supplement		Low supplement	
Serum total protein conc. (independent variable) at mating and:	3-4 yr	5-6 yr	3-4 yr	5-6 yr
Final BCS at mating	-0.5346	+0.4199	-0.0661	+0.4548
Final body weight at mating	-0.4717	+0.5811	-0.2469	-0.3323

Number of observations = 10

In the case of body weight gain these were -0.4717 and -0.2469 for younger does and +0.5811 and -0.3323 for older does in the HS and LS groups respectively. This implies that increasing level of mean serum total protein tended to be associated with decreasing BCS and body weight. Perhaps this was lipostatic response to flushing and hence an indication of the measure of mental imprint of the poor nutritional status of the dry season and of the desire of the animals to return to that status. However, the 5-6yr does tend to demonstrate a faster apparent lipostatic response to flushing initially but did not suffer physically as much as the younger does in the end. This may explain the positive correlation between the mean serum total protein and body mass gains in the older does. Perhaps, generally the mental lipostatic desire was suppressed by the physiologic rebound response to flushing resulting in a positive improvement in body mass in the end. The overall positive balance between lipostatic and rebound responses to nutritional treatment was significantly ($P<0.05$) influenced by high supplementation and young age as was demonstrated in the BCS gains at mating, especially in the HS (3-4yr), with gain of 0.6 or 37.50%.

4.8.3 Relation between Mean Serum LH Levels at Mating and Metabolic and Physical Parameters.

The coefficient of correlation between mean serum LH level and the metabolic as well as the physical profile of the experimental does at mating is presented in Table 4.16.

4.8.3-1 LH and Insulin

The correlation between mean serum LH and insulin concentrations at mating was generally positive, with coefficients ranging from +0.4554 to +0.9758, and significantly ($P<0.05$) favored high supplementation and young age. The correlation coefficients were HS (+0.9758 and + 0.5874) v LS (+ 0.4554 and +0.4971) for younger and older does, respectively, at each supplementation level. Similar results with high supplementation were reported by Meza – Herrera *et al.* (2004). They indicated that by-pass protein supplementation increased serum insulin, but they gave no correlation tendencies. McCann and Hansel (1986) and Tanaka *et al.* (2004) showed that increased LH was associated with increased insulin concentrations. In addition insulin (Kosior – Korzecka *et al.*, 2006) and / or insulin dependent changes in glucose availability (Bucholtz, 2000) modulate LH pulse frequency.

The LS (3-4yr) does in this study displayed relatively higher mean insulin (25.189 v 14.47 µIU / ml; $P < 0.05$) than for the HS (3-4yr) counterparts. However the latter showed an overall stronger and significant correlation coefficient ($r = +0.9758$ v + 0.4554) than for LS (3-4yr). Perhaps there were great individual variations in the LH concentrations during the follicular phase (Freitas *et al.*, 2004). Moreover, only 40% of LS (3-4yr) does contributed significantly to the mean value of the group as against 80% in the HS (3-4yr).

Table 4.16 Coefficient of Correlation between LH, Metabolic/ Physical Profile of Does at Mating Accounted For By Supplementation Level and Age

Parameters Correlation between mean	High Supplement		Low Supplement	
Serum LH conc. (dependent variable) and mean:	3-4 yr n = 5	5-6 yr n = 5	3-4 yr n = 5	5-6 yr n = 5
Insulin concentration	+0.9758[a]	+0.5874	+0.4554	+0.4971
Glucose concentration.	-0.2171	-0.6771	-0.3029	-0.0700
Total protein concentration.	-0.2292	-0.1007	+0.7569	-0.4479
Gain in BCS	-0.4122	-0.1231	-0.2030	-0.1591
Gain in body weight	+0.3567	+0.2320	-0.8726[b]	-0.4115

n = n umber of observations P< 0.05 a, b = significant correlation

This means that the increase in insulin–dependent LH concentration due to rebound metabolism was spread over more individual does in HS (3-4) than in the LS (3-4yr) group. Therefore, despite the higher mean serum insulin levels of the LS (3-4yr) group, the HS (3-4yr) group of does gave higher reproductive response to the insulin–induced increase in mean serum LH concentration than LS (3-4yr), because of the differences in number of responding individuals in each group. Increase in LH pulsatility is a limiting factor in successful resumption of ovarian activity in most female animals post anestrus (Lucy, 2003). Moreover, nutrition may improve ovulation rate in a flock by reducing the number of females failing to ovulate especially where ovulation rate is very low (1.0 - 1.2) (McKenzie and Terrill, 1937). Therefore, with more individual does in group HS (3-4yr) having an overall nutritionally induced increase in serum concentration of LH at mating this group had a better chance for higher reproductive response to flushing than the other groups. This was actually evident in their eventual prolificacy of 2.14 kids/conception and 100% multiple birth.

4.8. 3-2 LH and Serum Glucose

Neither supplementation levels nor age seemed to have influenced the moderate and negative correlation between serum mean LH and glucose concentrations at mating. The negative correlation may suggest LH in circulation increased with uptake of serum glucose especially at the brain level. Ohkura *et al.* (2004) demonstrated that glucose availability regulates the GnRH pulse generator activity in the ruminant to fine tune pulsatile GnRH release, which may affect the secretion of LH from the anterior pituitary (Hafez, 1992). It has also been shown in male lambs that in the presence of estradiol and insulin, changes in glucose availability modulate LH (GnRH) pulse frequency (Bucholtz *et al.*, 2000). Thus the LS (5-6) group which had highest serum glucose level (2.67 mmol/l), implying lowest uptake of glucose, also had significantly the lowest mean serum LH concentration of 0.212 mIU/ml, and hence lowest fertility rate of 67.6%.

4.8.3-3 LH and Body Mass Change

Except for the positive correlation coefficients for body weight gain in the high supplemented does, mean serum LH concentration at mating correlated negatively with both BCS and body weight gains in all the treatment groups, but more significantly ($P < 0.05$) in the younger does (Table 4.16)

In general, the mean serum level LH tended to decrease with increase body mass, especially in the 3-4yr does, except with high supplementation when LH tended to increase with body mass gain. This trend of relationship between LH and body mass gain seems to agree with the observation that alterations in pattern of gonadotropin secretion in heifers fed on low energy content are dependent on direction of body weight change (Roberson *et al.*, 1991). The experimental animals in this study tended to exhibit rebound –lipostatic – rebound metabolic response to flushing, with fluctuating body mass gains, which appeared to be stabilized with adjustment to high supplementation over the period. Thus the 3-4yr does, which had greater net positive lipostatic – rebound metabolism effects also had the highest mean serum levels of LH at mating (9.63 and 14.09 mIU/ml, P>0.05in the HS and LS groups respectively). Practically this may suggest that with approach of the breeding season fluctuation in the nutritional status need be avoided and breeding does fed to keep a reproductive fitting but not fat condition before mating (Hart *et al.*, 1999; Kinne, 2004; Nix, 2004).

4.8.4 Relation between Prolificacy, Age, and Physical Status at mating

Fig 4.6 shows the distribution of prolificacy in the entire experimental flock according to age, as well as body condition score and weight at mating. Generally, the curves approach a sinuous parabola with double optima points, suggesting that prolificacy tended to rise with age and body weight to an optimum and then declined only to rise again. The tendency for parabolic relationship between prolificacy and age as well as body mass is in agreement with Devendra and McLeroy (1992), Hafez (1993), Peacock (1996) and Kinne (2000).

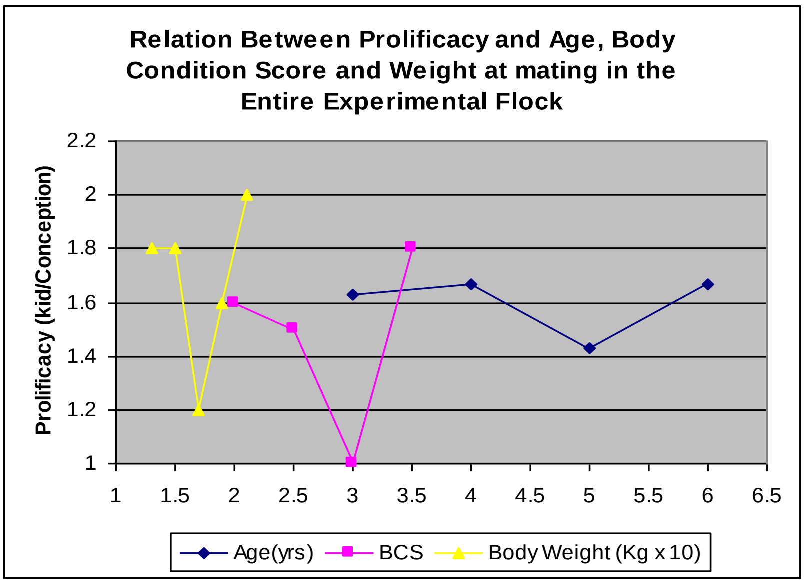

Fig.4.7 Relation between Prolificacy and Age, Body Condition Score and Weight at Mating In the Entire Experimental Flock

The pattern may be due to the differences in the 3-4yr and 5-6yr does in responding to the flushing treatments. The 3-4yr does, which were perhaps, in their prime of reproductive life exhibited more positive metabolic responses than the 5-6yr ones; and had propensity to be more fertile and prolific. The reproductive performance of does in their prime of life is generally higher than for does below or above the prime-age because the fertility of the latter is reduced by many physiological and environmental factors (Kinne, 2000) that may result in reduced ovulation rate and higher reproductive failures (Hafez, 1992). However, it appears individual differences altered this general observation and raised the prolificacy of the 5-6yr does in this study. Three six-year old does in the HS group, with a pedigree of repeated twinning had responded positively to flushing. Two kidded underweight (average=0.95kg) twins that died postnatal and one aborted twin fetuses close to term. This increase in twin-pregnancies together with the accompanied improvement in the body measurements of these aged does at mating influenced the distribution of prolificacy (liter size/conception) in the entire experimental flock to approach a second optimum at older age and higher body weight/ condition score (Fig 4.6).

That the litter biomass of these aged does were all lost perinatally suggests that while some aged (6yr) does may have the physiological potential to respond positively to flushing age-6 may not be economically prudent for nutritionally induced improvement in reproductive rate in goats. Therefore, economically, the age, body condition score and body weight at mating for optimum prolificacy in this study appear to be between 4 and 5 years, 2.0 and 2.5, and 15.0 and 17.0kg, respectively. Physiologically, however, body scores 3.0 to 3.5 corresponding to body weight 21kg may favor high prolificacy in some heavy does above prime of life. Jollans (1960) and Oppong-Anane (1975) observed in Djallonke sheep that twinning rate increased up to 3 and 4 years and fell. Landim goats of Mozambique have maximum twinning percentage at the third parturition (about the fourth and fifth years) (McKinnon and Rocha, 1985). Concerning body mass Blasu (1981) found that an optimum for Djallonke ewes may fall between 2.5 and 3.0 BCS and body weight of 22 to 27kg. However, goats were observed not to be as effective in feed utilization (ILCA, 1987) nor lipogenesis (Peacock, 1996) as sheep. Therefore, for the small size WAD does a score range of 2.0 to 2.5 and body weight of 15kg to 17kg may be considerably average enough under intensive/semi-intensive farm (Agricultural Station) management; though for traditional (extensive/free range) farm systems the ranges may be slightly higher due to free access to varied nutritional options.

4.9.0 Summary, Conclusions and Recommendations

4.9.1 Summary

In a bid to determine the link between nutrition and reproduction, the effects of two levels of a local feed supplement intake on the physical, metabolic and gonadotropin status of West African Dwarf WAD (goat) does at breeding were studied in a flushing experiment. The levels of supplement feed provided about 60% (High) and 20% (Low) of the daily dry matter requirement of goats. Forty WAD does aged between 3 and 6 years (inclusive) were grazed on grass (*Panicum maximum*) pasture and randomized within these supplementation levels as 3-4yr and 5-6yr groups, resulting in a 2x2 factorial of randomized complete block design. The parameters measured included body condition score, body weight, serum levels of total protein, glucose, calcium, insulin, gonadotropins (FSH and LH), fertility rate and prolificacy. In addition, the reproductive implications of interactions among these parameters were investigated. The following results were obtained:

At the end of 45days of flushing physical body measurements were improved due to high supplementation and age 3-4yr. Low level supplemented does gained an average of 13.26% and 7.20% body weight to attain average weights at mating of 16.19kg and 18.76kg for the 3-4yr and 5-6yr does, respectively. The corresponding values for their counterparts in the high supplementation were 12.48% and 9.03% resulting in final weights of 16.95kg and 19.08kg respectively. These changes improved the mean experimental flock body weight by 10.25% from 16.09±2.53 to 17.75±2.20. Similarly body condition scores, in the same order, were 17.64% and 16.90% gains resulting in final scores at breeding of 2.00 and 2.28; and 38.75% and 23.33% resulting in similar final score of 2.22 for 3-4yr and 5-6yr does in both the low and high supplement groups. The mean flock BCS was, therefore, improved by 23.86% to 2.18. These differences indicate the extent to which the physical body of does that have had a low dry-season-influenced nutritional status can be improved with high supplementation especially in does that are in their prime of life (3-4yr).

The metabolic profile at mating measured as mean serum levels of glucose, total protein, calcium, and insulin were affected by supplementation and age. The combination of high supplementation and 3-4yr age was associated with higher serum levels of total protein and calcium, but lower serum glucose levels – implying higher glucose utilization, protein synthesis and calcium assimilation. This profile appears to result from the interaction of two main factors: (1) a possible psychological adaptation to poor nutritional status over the long dry season and (2) a physiological response involving an apparent alternation of rebound metabolism and lipostatic reactions to flushing. In addition mean serum insulin levels at mating seemed to have been influenced by current increased metabolism rather than reserve nutritional status, the low supplemented 3-4yr does, with the greatest rebound-metabolism-responses in the last week of flushing having the highest mean insulin level (25.19±16.17mIU/ml). Goats, like other livestock, can adapt to their nutritional environment as a self-imposed regulation against food-insecurity, but can also respond to improved plane of nutrition with insulin-mediated rebound metabolism.

Concerning the pituitary functioning status at mating either the predetermined blood sampling time and frequency or insensitivity of the human assay kits seemed to have affected detection of FSH. The mean serum concentrations of LH were not statistically affected by treatment. However both the higher average detectable FSH (18.99mIU /ml) and LH (14.05mIU/ml) were obtained in the low supplemented 3-4yr does that showed the greatest rebound metabolism in the last week of flushing. These observations imply that current increase in metabolism rather than reserve nutritional status affects immediate pre-breeding (follicular phase) function of the anterior pituitary appreciably and more so in 3-4yr does.

With reproductive performance fertility was not affected by age or supplementation statistically, but there was clear tendency to favor 3-4yr low–supplemented does, perhaps due to their higher metabolic-reproductive endocrine functioning status at mating. Prolificacy was highest in the high supplemented 3-4yr does (2.14 kid/conception) that had the highest physical and metabolic improvements at mating, raising the flock kidding performance by 29.0% to 1.65 kids/conception. Flushing may be more effective in influencing reproductive performance of goats when it promotes **both** rapid metabolism and physical body improvement.

Various levels of correlation were found among the treatment effects due to supplementation and age on the physical, metabolic, endocrinologic and reproductive profiles of the experimental does. Due to its role in glucose and amino acid utilization insulin tended to correlate with physical body development and metabolic profile of the does both positively and negatively; the direction of the correlation seemed to have been influenced by age and previous nutritional status. Thus insulin correlated negatively with body weight gain in 5-6yr but positively in 3-4yr does; the correlation with gain in BCS was negative in high but positive in low supplemented does; insulin correlated positively with total protein in almost all the treatment groups (except one); and insulin correlated with glucose levels negatively in 3-4yr, but positively in 5-6yr does. These directions of correlation together suggest insulin’s orexigenic regulation of physical and metabolic development of does prior to breeding. In addition, the correlations also show that during follicular phase insulin level did not only increase but was associated with reducing serum glucose level and rising serum total protein levels in the 3-4yr does. This, alongside the possibility that the 3-4yr does were in prime of life, indicate that flushing may increase sensitivity to insulin metabolic actions in 3-4yr does, resulting in increased GnRH and gonadotropin levels, ovarian activity, behavioral estrus, fertility and high prolificacy.

Body mass gains correlated positively with increasing serum total protein levels in 5-6yr does but negatively in 3-4yr does. These relations seem to reveal high incidence of lipostatic tendencies among the experimental animals and how both psychological and physiological adjustment to high supplementation for at least six weeks can reverse the tendency before breeding.

There was positive correlation between LH and insulin in this study. This indicated an advantage of high–supplementation by distributing the increase of insulin – dependent LH in many more individuals and so enhanced the flock reproductive performance. LH correlated also with body weight, but this tended to be influenced by the direction of weight change. The practical implication is that there is need to avoid fluctuations in body mass of animals by feeding to keep them in a reproductively fit condition before mating.

Graphical presentation reveals that prolificacy tends to exhibit parabolic relations with age, and with body mass at mating. The possible optimum age, BCS and body weight at mating for the small size WAD does may be 4 years, 2.0 – 2.5, and 15 -17 kg, respectively.

The results of this study corroborate the hypothesis that flushing causes an overall rapid change in metabolism favoring protein synthesis and glucose utilization; and that this leads to high gonadotropin and ovarian activity that raises the probability of multiple ovulation and prolificacy. It also corroborates the indication that insulin may be a typical metabolic hormone that links nutrition to reproduction. The physiologic mechanisms of insulin’s function along the nutrition - reproductive axis appear to involve its chemostatic nature as a hormone with both metabolic and reproductive signaling at both the brain and ovarian levels. Increased nutritionally induced bioavailability of insulin, alongside its orexigenic and energy homeostatic signaling in the arcuate nucleus, may stimulate increased GnRH and subsequent increase in gonadotropin (FSH and LH) secretion into circulation.

4.9.2 Conclusions and Recommendations

1. The physical body condition and weight of 3-4yr does that are in prime of life and had a low nutritional status as may be caused by dry season feed scarcity can be improved with high energy/protein supplementation.
2. Goats, like other livestock, can adapt to their nutritional environment as a self-imposed regulation against food-insecurity, but can also respond to improved plane of nutrition with insulin-mediated rebound metabolism.
3. Current increase in metabolism rather than reserve nutritional status affects immediate pre-breeding (follicular phase) function of the anterior pituitary appreciably and more so in does with growth potential.
4. Nutritional flushing may be more effective in influencing reproductive performance of goats when it promotes **both** rapid metabolism and physical body improvement.
5. Nutritional flushing may increase sensitivity to insulin metabolic actions in growing does, resulting in increased GnRH and gonadotropin levels (per individual and number of individuals), ovarian activity, behavioral estrus, fertility and high prolificacy.
6. West African Dwarf (WAD) does responded positively to luteolytic estrus synchronization with $PGF_{2\alpha}$ and flushing with high energy/protein supplementation for six weeks at the end of a long dry season, in a semi-arid or transitional ecozone in Ghana. The probable optimum age, BCS and body weight at mating for improving the prolificacy of does were 4yr, 2.0–2.5 and 15-17kg, respectively. Flushing improved BCS, body weight and prolificacy of WAD does by 23.86%, 10.25% and 29.0%, respectively. The study results also corroborated suggestion of insulin as possible metabolic-reproductive hormone.

However, these findings need further verification in other parts of the breeding year, ecozones of Ghana, and traditional (free range or extensive) goat farming system. Season, climate and management can affect the nutritional status of the doe in relation with reproduction. Blood sampling protocol for gonadotropin assay may start earlier than 31h after estrus-inductive administration of $PGF_{2\alpha}$ since pre-estrual/ovulatory FSH levels apparently reduced to undetectable levels by 31h after the prostaglandin treatment in this study. Serum lipid and leptin levels must be included in blood chemistry and hormone analyses to verify lipostatic status of animals and the relation between leptin and insulin during flushing in affecting ovulation rate and prolificacy. For effective flushing with feed supplement 5-6 yr does may not be included in the breeding stock for fear of not producing thrifty litters. Finally, programmed breeding and regular body condition scoring are recommended for the breeding and replacement stock. This is to enable identification and effective management of their nutritional needs that will keep them in fitting body condition - between moderate (score 2) and good (score 3) – for optimal reproductive rate.

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