SLOVAK UNIVERSITY OF AGRICULTUR IN NITRA

FACULTY OF AGROBIOLOGY AND FOOD RESOURCES

EFFECT OF GLYCEROL AS AN ENERGY SUPPLEMENT IN NUTRITION ON EWES REPRODUCTION

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Nitra 2011 **Abdulhadi Omar Qoja**

DECLARATION ON OATH

Signed Abdulhadi Omar Qoja declare that I have a dissertation topic (Effect of glycerol as an energy supplement in nutrition on ewes reproduction) prepared separately using the literature. I am aware of the legal consequences if the information was false.

Nitra 16.12.2010

Abdulhadi Omar Qoja

DEDICATION:

I dedicate this thesis to my wife "Shan" Your love and support gives me inspiration and courage. I could not have done it without you, which she gave me the motivation to seek a higher education and for this I will be indebted to her for the rest of my life.

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ABSTRAKT:

V práci sme skúmali vplyv glycerolu ako náhradného zdroja energie (pokus I) a ako doplnok energie (pokus II) vo výžive bahníc krížencov plemien IDF a Merino na reprodukčné parametre, hormonálny profil, metabolický profil a krvný obraz počas sezóny párenia. Do pokusu I bolo vybratých 48 oviec (vek 4-7 rokov, hmotnosť 58-73 kg), ktoré boli náhodne rozdelené do 4 skupín po 12 oviec v každej skupine. Kŕmna dávka (KD), ktorá sa začala podávať 1 mesiac pred sezónou párenia bola rozdelená podľa obsahu energie do troch úrovní. 1. skupina dostala KD s obsahom 100% energie z prírodných krmív, 2. skupina dostala KD so 70% energie a 3. skupina KD s 80% energie z prírodných krmív a 20% energie vo forme glycerolu. Kontrolná skupina bola na pastve a prijímala krmivo ad libitum počas experimentu. Do pokusu II bolo zaradených rovnako 48 oviec vo veku 5-7 rokov v hmotnosti 62-75 kg. Ovce boli náhodne rozdelené do 4 skupín, v každej 12 oviec. Kŕmna dávka oviec obsahovala kukuričnú siláž (0, 5 kg/ovca/ deň), lúčne seno (0,8 kg/ovca/deň) a lucernové seno (0,4kg/ovca/deň).ku KD sa pridávalo 1.,2. a 3. skupine glycerol (1.sk. 150 ml, 2.sk. 100ml a 3.sk. 50ml./ovca /deň) rozriedený s vodou (5l /ovca/deň). 4. skupina nedostala prídavok glycerolu. V prvom experimente 20% glycerolu spôsobilo signifikantne (P< 0,05)skrátenie dĺžky ruje, zatiaľ čo % a dĺžka gravidity, počet jahniat, počet dvojičiek, počet baránkov, oplodnenosť a celková plodnosť boli vyššie. Unifetálnych pôrodov bolo signifikantne menej, pričom sa relatívne zvýšil pomer jahničiek a ich hmotnosť (P< 0,05). Rovnako sa zvýšila aj celkovo hmotnosť jahniat z experimentálnych skupín (P< 0,05). Na začiatku a na konci experimentu (obdobie gravidity) sa v pokusných skupinách nesignifikantne znížila koncentrácia FSH rovnako sa nepreukazne znížila vo fáze estru koncentrácia LH (P< 0,05). Koncentrácia progesterónu bola signifikantne vyššia na začiatku a čase ruje. V gravidite bola vyššia nesignifaktne. Vplyv glycerolu na prolaktín však nebol jasný. Neovplyvnil ani ALP a celkové bielkoviny, hoci sa zistili signifikantné rozdiely medzi skupinami (P<0.05), keď boli preukazné rozdiely v hodnotách ALT a AST so znížením ich hodnôt v štádiu gravidity. Hodnoty urey a glukózy sa zistili nepreukazne vyššie nazačiatku pokusu a znižovali sa v gravidite. Hladiny cholesterolu boli v dvoch fázach pokusu nižšie, hoci na rozdiel od toho sa zistili hodnoty triglyceridov preukazne vyššie na začiatku experimentu a znížili sa v gravidite. Hodnoty leukocytov boli na začiatku experimentu v pokusných skupinách nepreukazne

nižšie. Počet LY bol na rozdiel od gravidity vyšší (P<0.05) na rozdiel od granulocytov, ktoré mali opačnú tendenciu. Na začiatku pokusu a v čase ruje sa zistili nepreukazne vyššie hodnoty ER, HGB a HCT.

V druhom experimente bol glycerol použitý ako doplnok energie v rôznej úrovni. Pri 30% glycerolu v kŕmnej dávke sa zistila preukazne kratšia ruja (P<0.05). Vo všetkých troch pokusných skupinách sa nepreukazne zvýšila gravidita, ale dlhšia bola iba pri doplnku 10% glycerolu. Pri 30% doplnku sa preukazne zvýšil počet jahniat (P<0.05). Vo všetkých troch pokusných skupinách sa zvýšil počet jahniat, dvojičiek a trojičiek Pri 10% doplnku glycerolu bol väčší podiel barančekov aj ich hmotnosť bola väčšia. Na rozdiel od toho sa pri 20% doplnku narodilo viac jahničiek a pri 30% doplnku glycerolu bola preukazne vyššia hmotnosť jahničiek ako aj celková hmotnosť. Vo všetkých experimentálnych skupinách bola vyššia plodnosť a pri 30% doplnku bola vyššia aj oplodnenosť. V skupine s 30% doplnkom glycerolu bola vyššia koncentrácia progesterónu na začiatku pokusu a v gravidite. Pri 20% doplnku sa zistili nižšie koncentrácie estradiolu. Pri 10% doplnku bola hladina LH nižšia na začiatku experimentu, pri 30% na rozdiel od toho v gravidite. Rovnaký vplyv sa prejavil na začiatku experimentu aj na prolaktín, ktorého koncentrácia stúpla v gravidite. V skupine s 30% doplnkom glycerolu bola zistená preukazne zvýšená hladina ALP a vyššia hladina ALT a AST aj na začiatku aj na konci experimentu. Pri 20% doplnku sa zistili vyššie hladiny celkových bielkovín na rozdiel od glukózy a urey. Pri 20 % bola zistená preukazne nižšia koncentrácia urey a a glukózy na začiatku pokusu, ale pri 30% sa prejavil pokles urey a glukózy v gravidite. Koncentrácia cholesterolu bola vyššia pri 10% doplnku na začiatku pokusu, ale pri 30% doplnku v gravidite. Hladina triglyceridov bola vyššia pri 10% doplnku v oboch testoch. Hladina Na bola nižšia pri 20% na začiatku pokusu zatiaľ čo pri 30% doplnku v gravidite. Hladina Cl mala úplne opačnú tendenciu. Pri 20% doplnku sa zistili vyššie koncentrácie K počas celej doby sledovania. Koncentrácia Ca boli vyššie pri 20% doplnku na začiatku a pri 30% doplnku nižšie v gravidite, zatiaľ čo hladiny P boli v oboch testoch vyššie. Úroveň Mg bola nižšia pri 30% na začiatku a pri 20% v gravidite. Počty Le boli pri 30% doplnku nižšie na začiatku a vyššie v gravidite. Pri 20% doplnku bol zistený vyšší podiel LY a menší GR na začiatku. Pri 30% doplnku boli preukazne nižšie hodnoty LY a vyššie GR v gravidite. Pri 30% doplnku sa zistili vyššie počty erytrocytov aj na začiatku pokusu aj v gravidite. Pri 20%

doplnku sa zistili vyššie hodnoty HGB a HCT na začiatku pokusu, pri 10% doplnku boli tieto vyššie v čase gravidity.

Kľúčové slová: ovce, glycerol, výživa, reprodukcia, hormóny, metabolizmus, hematológia

ABSTRACT:

In this work we investigated the effect of glycerol as replacement energy (trial I), and as supplement energy (trial II) in the diet of cross-bred ewes (IDF X MERINO) on (reproduction characteristics, hormones, metabolic profile and haematological properties) in breeding season. In trial (I) Forty eight ewes (4-7 years of age, 58-73 kg body weight range) were selected and divided randomly into 4 groups of 12 ewes in a completely randomized design. Three different levels of energy that content a feed ration by (NRC) requirement of ewes that given one month before the breeding season to three groups respectively, 1st group (100% forage energy), 2nd group (70% forage energy) and 3rd group (80% forage energy + 20% glycerol energy), control group was grazed only on pasture by adding libitum during experiment period. In trial (II) Forty eight ewes (5-7 years old, 62-75 kg body weight) were selected and divided randomly into 4 groups of 12 ewes each in a completely randomized design. The animals of trial (II) were fed on corn silage (0.5 kg\day\ewe), meadow hay (0.8 kg\day\ewe) and alfalfa hay (0.4 kg\day\ewe). The trial treatments were: groups 1st, 2nd and 3rd with 150, 100 and 50 ml glycerol\ewe\day respectively which provided diluents with water (5 litter\ewe\day), and 4th group (control) without glycerol. The samples of blood were collected at the start of experiment, beginning of estrus cycle (for hormonal and haematology evaluations) and at the end of experiment for trial (I), also at the start and at the end of experiment for trial (II). For the first trial which used glycerol as alternative source of the food energy (20%) supply, feeding glycerol in diet during breeding season was effected relatively on estrus length hours which decreased significantly ($P<0.05$), while percentage of gravidity, pregnancy length, number of lambs, twins lambing, male lambs, fertility, prolificacy and fecundity were increased insignificantly. Single lambing was decreased insignificantly, whereas female lambs and female lambs weight were affected by glycerol relatively that increased significantly ($P<0.05$), while glycerol increase total lambs weight significantly ($P<0.05$). Glycerol was decreased FSH insignificantly at the start and at the end (pregnancy period) of trial, while decreased LH in estrus phase insignificantly. Progesterone was higher in two stages (at the start and estrus phase) significantly ($P<0.05$). In pregnancy period was higher insignificantly. Glycerol increase estradiol at the start and estrus phase then decrease in pregnancy period insignificantly. Glycerol effect on prolactin was not clear. Glycerol did

not effected ALP and total protein clearly although found significant ($P<0.05$) differences between groups, while glycerol effected ALT and AST significantly ($P<0.05$) which decreased in pregnancy period. Glycerol effected urea and glucose insignificantly which increased at the start of trial and decreased in pregnancy period. Cholesterol affected by glycerol insignificantly that decreased in two stages of trial, while triglyceride affected by glycerol significantly ($P<0.05$) which increased at the start of trial and decreased in pregnancy period. Glycerol decrease Na concentration in two stages of trial insignificantly. Glycerol effected on Cl and Mg concentrations significantly ($P<0.05$) which decreased in two stages of trial, glycerol effect on K, Ca and P concentrations was not clear although found significant ($P<0.05$) differences between groups. WBC affected by glycerol insignificantly ($P<0.05$) that decreased at the start of trial. Glycerol increase percentage of LY at the start of trial insignificantly and at pregnancy period was decreased significantly ($P<0.05$), conversely than glycerol effect on GR percentage which decrease at the start of trial insignificantly and increase in pregnancy period significantly ($P<0.05$). Glycerol increase RBC, HGB and HCT in two stages (at the start and estrus phase) of trial insignificantly.

In second trial glycerol was used as supplement energy to the food energy with different levels. 30% glycerol supply in diet decrease estrus length significantly ($P<0.05$). Glycerol supplementation with three levels increased gravidity insignificantly, in pregnancy length glycerol 10% was higher insignificantly than others. 30% glycerol increase number of lambs significantly ($P<0.05$). Glycerol levels (10%, 20% and 30%) increase insignificantly single, twins and triplet lambing respectively. Glycerol 10% increase male lambs and male lambs weight insignificantly. Glycerol 20% increased female lambs insignificantly. Glycerol 30% increased female lambs weight and total lambs weight significantly ($P<0.05$). Glycerol with three levels was increase fertility insignificantly, while glycerol 30% increases prolificacy and fecundity insignificantly. Using glycerol 30% as energy supplement increase FSH and progesterone concentrations in both stages of trial (at the start and pregnancy period) insignificantly. 20% glycerol decrease insignificantly estradiol concentrations in two stages of experiment. Glycerol 10% decrease LH concentrations at the start of experiment while glycerol 30% decrease LH in pregnancy period insignificantly. 30% glycerol effect was insignificantly on prolactin hormone which

decreased at the start of experiment and increased in pregnancy period. Glycerol 30% effect on enzymes profile was higher significantly ($P<0.05$) in ALP and higher insignificantly effect on ALT and AST in both stages of trial. Glycerol 20% increase total protein in two stages of experiment, as regard to urea and glucose. Glycerol 20% decrease urea significantly ($P<0.05$) and glucose insignificantly at the start of trial, whereas glycerol 30% decrease urea significantly ($P<0.05$) and glucose insignificantly in pregnancy period. Cholesterol was insignificantly higher in glycerol 10% treatment at the start of trial and in glycerol 30% treatment in pregnancy period. Glycerol 10% increase triglyceride insignificantly in both stages of experiment. Na affected by glycerol treatments insignificantly which decreased in glycerol 20% treatment at the start of trial and increased in glycerol 30% treatment in pregnancy period. Glycerol affected insignificantly on Cl in both stages of experiment which increased at the start of trial and decreased in pregnancy period. 20% glycerol increase K significantly ($P<0.05$) at the start of trial and insignificantly in pregnancy period, Ca concentrations were higher in glycerol 20% treatment at the start of trial and lower in glycerol 30% treatment in pregnancy period insignificantly. Glycerol 20% increase P insignificantly in both stages of experiment. Mg was lower significantly in glycerol 30% treatment at the start of trial then decreased in glycerol 20% treatment in pregnancy period insignificantly. WBC affected by glycerol 30% insignificantly which decreased at the start of trial and increased in pregnancy period. 20% glycerol effected insignificantly at the start of trial which increased LY and decreased GR. Glycerol 30% effected significantly ($P<0.05$) in pregnancy period which decreased LY and increased GR. RBC affected by glycerol 30% insignificantly which increased in both stages of experiment. Glycerol 20% increased both of HGB and HCT insignificantly at the start of trial, whereas glycerol 10% increases both of HGB and HCT in pregnancy period insignificantly.

Keywords: Ewes, glycerol, nutrition, reproduction characteristics, hormones, biochemical parameters, haematological properties.

ABBREVIATION:

AI	-Artificial Insemination
ALP	-Alkaline Phosphatase
ALT	-Alanine Transaminase
ART	-Assisted Reproductive Technology
AST	-Aspartate Transaminase
BCS	-Body Condition Score
Ca	-Calcium
Cl	-Chlorine
CL	-Corpus Luteum
CNS	-Central Nervous System
CRD	-Completely Randomized Design
DHA	-Docosahexaenoic Acid
DM	-Dry Matter
DMI	-Dry Matter Intake
EPA	-Eicosapentaenoic acid
FDA	-Food and Drug Administration
FO	-Fish Oil
FSH	-Follicle Stimulating Hormone
GnRH	-Gonadotrophin-Releasing Hormone
GR	-Granulocyte
HCT	-Hematocrite
hCG	-human corticoid gonadotrophin
HGB	-Hemoglobin
Ib	-Pound
IGF-I	-Insulin-like growth factor 1
IDF	-I Le de France
K	-Potassium
LH	-Luteinizing Hormone
LY	-Lymphocyte
Mcal	-Mega Calorie
Mg	-Magnesium
MJ	-Mega Joule
Na	-Sodium
NEB	-Negative Energy Balance
NEL	-Net Energy of Lactation
NRC	-National Research Council
P	-Phosphor
PDI	-Protein Digest intestine
pFSH	-Porcine Follicle Stimulation Hormone
PG	-Propylene glycol
PGF2α	-Prostaglandin F2α
PMSG	-Pregnant mare Serum Gonadotrophin
RBC	-Red blood Cell

SPSS	-Statistical Package for the Social Science (computer program for statistical analyses using)
SUA	-Slovak University of Agriculture in Nitra
UMMB	-Urea Molasses Mineral Block
US	-United States
VFA	-Volatile Fatty Acid
WBC	-White Blood Cell
xrpm	-Rotary per minute

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1. INTRODUCTION:

Domestic sheep (Ovis aries) are ruminant mammals typically kept as livestock. Like all ruminants, although the name "sheep" applies to many species, in everyday usage it almost always refers to (Ovis aries). Domestic sheep is the most numerous species in their genus, numbering a little over 1 billion, and are most likely descended from the wild mouflon of Europe and Asia. One of the earliest animals to be domesticated for agricultural purposes, sheep is raised for fleece, meat (lamb or mutton) and milk. A sheep's wool is the most widely used of any animal. Sheep continue to be important for wool and meat today, and are also occasionally raised for pelts, as dairy animals, or as model organisms for science, and sheep follow a similar reproductive strategy to other herd animals. A group of ewes is generally mated by a single ram, which has either been chosen by a breeder or has established dominance through physical contest with other rams (in feral populations). Most sheep are seasonal breeders, although some are able to breed year-round. Ewes generally reach sexual maturity at six to eight months of age, and rams generally at four to six months. Ewes have estrus cycles about every 17 days, during which they emit a scent and indicate readiness through physical displays towards rams.

The sheep are a seasonally poly estrus animals, it multiple sessions leering but in certain seasons of the year. Characterized by high fertility, where in this case the ovarian physiology will be active, the seasonal breeding for sheep is one of the adaptation method for wild sheep familiar with the surrounding environment as natural to reproduce birth lambs correspond to the natural cycle of growth of pasture plants in those areas where the presence lambs in availability season of green pastures.

Nutrition plays a major role in the overall productivity, health, and well-being of the sheep flock. Because feed costs account for approximately two-thirds of the total cost of production on most sheep farms, it is important that producers consider nutrition management a top priority. Nutrient requirements of sheep vary with differences in age, body weight, and stage of production. The five major categories of nutrients required by sheep are; water, energy, protein, vitamins and minerals. During the grazing season, sheep are able to meet their nutrient requirements from pasture and a salt and mineral supplement. Hay is provided to the flock when forages are limited, and grain may be added to the diet at certain stages of production when additional nutrient supplementation is required. Small

grain pastures or stockpiled fescue can supply up to one-half of the feed requirements of the ewe flock during the winter. For winter-born lambs, creep diets and diets for early-weaned lambs are formulated from high energy feed grains and protein supplements to promote accelerated growth. During the grazing season, pastures of mixed grass and clover, alfalfa, small grain, and turnip serve as excellent sources of nutrition for growing lambs. A source of clean, fresh water is provided to sheep at all times.

Good nutrition is the essential factors to ensure better production. The best food quality increases animal production, both in terms of meat, milk and able of births to sound infrastructure capable of growing and to reach the production stage. And the provision of food for animal is a balanced supply of animal energy required for operations essential for the body, including reproduction. And productivity depends on reproductive efficiency and is often measured by the number of offspring per breeding animal per unit of time.

Insufficient energy limits performance of sheep probably more than any other nutritional deficiency. An energy deficiency may result from inadequate amounts of feed or from feeds (generally forages) that do not contain enough protein to sufficiently "unlock" the energy in the feedstuff. Energy deficiencies can cause reduced growth rate, loss of weight, reduced fertility, lowered milk production, and reduced wool quantity and quality.

Reproductive efficiency depends largely upon proper nutrition before and during the breeding season. Large-bodied ewes tend to produce more lambs per ewe. Do not confuse ewes of large size and scale with ewes that look large because they are fat. Usually, excessively fat ewes have lower conception rates and higher embryonic mortality. Furthermore, extremely poor body condition is not conducive to efficient fertility and reproductive performance.

Fertility is one of the most important determinants of success in breeding sheep, in terms of the number for mature eggs from the ovaries produce during the Leering (ovulation rate), where it is the main factor for the number of births resulting from the herd and thus the amount of meat resulting from such births. The greater number of lambs for ewe led to less production cost per lamb in the farm.

2. LITERATURE REVIEW.

2.1 REPRODUCTION IN SHEEP.

Sheep follow a similar reproductive strategy to other herd animals. A flock of ewes is generally mated by a single ram, which has either been chosen by a farmer or has established dominance through physical contest with other rams (in feral populations). Most sheep are seasonal breeders, although some are able to breed year round. Reproduction in sheep is influenced by numerous factors. These include: genetic potential, nutritional status, environmental factors, day length or photoperiod effects, health status and other factors. These factors are important in both the ram and the ewe. Because many sheep have the potential for multiple births we can use management practices to influence these factors and to increase the reproductive rate.

2.2 PUBERTY AND SEXUAL MATURITIY.

Puberty is a basically the result of a gradual adjustment between increasing gonadotropic activity and the ability of the gonads to simultaneously assume steroid-genesis and gametogenesis (Hafez and Hafez, 2000).

Puberty is influenced by age, breed, genetic selection, body size, nutrition, and season of birth. Most ewe lambs reach puberty between 5 and 12 months of age. Ewe lambs will tend to reach puberty their first fall. For this reason, spring-born ewe lambs tend to exhibit puberty earlier than fall-born ewe lambs. Lambs born early in the season reach puberty earlier than those born late in the season, due to their increased age and body weight. High levels of feed pre- and post-weaning reduce the age at puberty. Single lambs cycle at a younger age than twin and triplet-born ewe lambs, due to their size advantage. Ewe lambs from fine-wool, coarse wool, and late-maturing medium-wool breeds reach puberty later than many of the meat (Suffolk, Dorset, etc.) and hair sheep (Katahdin, St.Croix, and Barbados Blackbelly) breeds. Finnsheep and Romanov ewe lambs and their crosses reach puberty at an early age. Crossbred ewe lambs cycle at a younger age than purebred ewe lambs. (Schoenian, 2005).

Maturation of the hypothalamo-pituitary-gonadal axis is affected by the interaction between environmental and genetic factors and depends on the coordinated functions of luteinizing hormone (LH) and follicle stimulating hormone (FSH). Thus, the gonadotroph

population is the morphological setting for the endocrine mechanism that prepares the reproductive system for puberty and then for sexual maturity in the mammal (Wankowska and Polkowska, 2006).

2.2.1 CLIMATE.

Animal environment is affected by climatic factors that include temperature, humidity, radiation, and wind. Extremes in climate alter energy transfer between the animal and its environment and can affect deleteriously reproduction. Seasonal variation of environment alters estrus activity and duration of estrus. Conception rates are reduced under stress of heat and cold (Gwazdauskas, 1985). High temperatures are detrimental to fertility, embryo survival, and fetal development. This is the biggest objection to fall lamb production. High temperatures at breeding can reduce conception rate. Heat stress during gestation impairs fetal development and can cause lambs to be significantly smaller at birth.

2.2.2 TECHNOLOGY OF HOUSING.

Housing needs vary by climate, lambing season, and individual preferences. If lambing will occur during periods of inclement weather, more elaborate housing is generally required. If lambing will occur on pasture during periods of mild weather, simple shelters may be all that is needed. Lambing percentages are higher when shed lambing is practiced. Housed sheep have lower nutritional requirements. Sheep raised outside have fewer respiratory problems. All operations need facilities where they can store feed, bedding, and equipment. Hay stored in a barn or shed will maintain its quality better than hay that is stored outside, even if the hay is covered. Equipment will last longer if it is housed under roof.

There are many different types of housing that can be used for sheep. Traditional bank barns, pole buildings, and metal buildings are usually the most expensive, but they provide the best protection for the shepherd, sheep, and feed. A low-cost alterative to traditional housing is a greenhouse-type structure called a "hoop house." A hoop house has an arched metal frame that is covered with a heavy fabric. Fabrics last for approximately 15 years. Sheep facilities do not need to be built new. Old dairy and swine barns make good sheep raising facilities. Many facilities can be remodeled to accommodate sheep rising. Facilities

should be located on elevated, well-drained sites. When designing a three-sided shelter, the open side should face south away from the prevailing wind. The barn should be easily accessible for deliveries and manure handling. The site should allow for installation of water and electricity. When confined to a building, a bred ewe requires 12 to 16 square feet of space. Lambing pens should be 16 to 25 square feet in size. In group housing, a ewe with her lambs needs 16 to 20 square feet. Feeder lambs need 8 to 10 square feet. Less space is required if sheep are raised on slotted floors or if they have access to an exercise area or pasture. Shearing before housing will allow stocking rates in the barn to be increased by up to 20% (Schoenian, 2005).

2.2.3 HEALTH.

Disease and health problems of sheep are closely associated with management and nutrition. Medication cannot cure results of poor management and poor nutrition. The first step in controlling a disease problem is to identify the disease. Producers should seek professional help from a qualified veterinarian. Autopsies and accurate health records can be helpful in improving the overall health program. Any time drugs are administered to livestock, it is imperative that the drugs are used strictly as directed on the label, unless otherwise directed by a veterinarian.

2.2.4 MANAGEMENT.

Low productivity is a feature of traditional extensive systems of sheep production. The seasonal nature of production reduces the economic viability of the traditional flock. Therefore, more modern management systems must be associated with various levels of intensification, the success of which are determined to a large extent by the efficiency of reproductive management. Reproduction may be managed by improvement in productivity of the flock, general improvement of fertility, increased prolificacy and increased number of lambings per year (Ptaszynska, 2006). And poor nutritional management of the dairy cow, particularly before and after calving, is a key driver of infertility (Roche, 2006).

2.3 SEASONALITY.

One of the most important features of ovine reproduction is seasonality, though this is

not exclusive to sheep, of course. Reproduction follows a seasonal pattern in ewes, i.e. alternating periods of anestrus and sexual activity. In temperate regions, seasonality is regulated by the photoperiod, or daylight length (reducing daylight length stimulates sexual activity, and increasing daylight length induces anestrus). Sheep are therefore categorized as 'short day' breeders (Ptaszynska, 2006). Seasonal reproduction in sheep is mainly regulated by photoperiod. However, other cues from the environment (temperature, nutrition and social relationships) are believed to modulate its effect. While in temperate regions the photoperiod is the decisive factor and other environmental factors can only influence the onset and the duration of the anestrus period, in tropical areas nutritional level is probably responsible for some seasonal acyclicity (Rosa and Bryant, 2003). Ewes are able to 'monitor' changes in the daily photoperiod by the circadian secretion of melatonin from the pineal gland. Melatonin output is regulated by photoperiod and elevated concentrations are found in blood only during the hours of darkness (Rosa and Bryant, 2003). One important economic constraint of the sheep industry is the seasonal nature of ewe fertility (Notter, 2002). Seasonality results in widespread lambing during the winter and spring seasons followed by marketing of lambs in summer and fall (Casas et al., 2005).

2.4 REPRODUCTION AND HORMONES IN EWES.

The control of mammalian reproduction has shifted from the central nervous system (CNS) to regulation by two separate systems, the CNS and the endocrine systems, both of them regulate the function of the reproductive system. The endocrine system uses chemical messengers or hormones to regulate slow body processes, e.g., growth and reproduction. Hormone is a chemical substance synthesized and secreted by a ductless endocrine gland, which passes into the circulatory system for transport. Reproductive hormones are derived primarily from four major system or organs: various areas of the hypothalamus, anterior and posterior lobes of the pituitary gland, gonads (testis and ovary including their interstitial tissues and corpus luteum), and the uterus and placenta (Hafez and Hafez, 2000). Appropriate thyroid gland function and thyroid hormone activity are considered crucial to sustain the productive performance in domestic animals (growth, milk or hair fiber production). Changes of blood thyroid hormone concentrations are an indirect measure of the changes in thyroid gland activity and circulating thyroid hormones can be considered as

indicators of the metabolic and nutritional status of the animals. Thyroid hormones play a pivotal role in the mechanisms permitting the animals to live and breed in the surrounding environment. Variations in hormone bioactivity allow the animals to adapt their metabolic balance to different environmental conditions, changes in nutrient requirements and availability, and to homeorhetic changes during different physiological stages. This is particularly important in the free-ranging and grazing animals, such as traditionally reared small ruminants, whose main physiological functions (feed intake, reproduction, hair growth) are markedly seasonal (Todini,2007).

2.4.1 FOLLICLE STIMULATING HORMONE (FSH).

Follicle stimulating hormone is a glycoprotein that secreted from the anterior pituitary gland and stimulates the growth and maturation of the ovarian follicle or the Graafian follicle. Also it needs the presence of LH to stimulate estrogen production (Hafez and Hafez, 2000). The greater dietary intake was reported to increase FSH concentration in the late luteal phase and the follicular phase of the cycle and was associated with an increase in ovulation rate (Rhindet et al., 1985). Lane et al.,(2009) observed during the first transient FSH increase of the estrus cycle in heifers, there were clear indications that serum FSH was regulated by its own synthesis pathway, and perhaps by alterations in pituitary sensitivity to circulating steroids in response to changes in pituitary steroid receptor content, primarily estrogen receptor-α.

2.4.2 LUTEINIZING HORMONE (LH).

Luteinizing hormone is a glycoprotein that secreted from the anterior pituitary gland. Basal levels of LH act in conjunction with FSH induce estrogen secretion from the large ovarian follicle .The preovulatory surge of LH is responsible for rupture of the follicle wall and ovulation. LH stimulates the interstitial cells of both the ovary and testis (Hafez and Hafez, 2000). The pattern of gonadotropin secretion (LH and FSH) in ruminants changes according to the different reproductive stages (Caraty and Skinner, 1999). LH is stored in granules and its release is through a GnRH regulated pathway. In contrast, FSH is released predominantly via a constitutive pathway and the amount released is closely related to the rate of synthesis (McNeilly et al., 2003). Ciechanowska et al., (2008) found the LH

concentration in blood plasma of luteal phase ewes were significantly higher than those of anestrus ewes. Butler et al.,(2006) found the mean LH, and pulse amplitude was not different between control and propylene glycol cow groups.

2.4.3 ESTROGEN HORMONE.

Estrogens are steroid hormones that secreted by the ovary, testes, placenta, and adrenal cortex. Estradiol is the primary estrogen, with estron and estriol representing other metabolically active estrogens. Estradiol is the biologically active estrogen produced by the ovary with smaller quantities of estrone. Except for the possible secretion of small amount of estriol in the luteal phase of the cycle, most estriol and related urinary estrogens is metabolic breakdown product of secreted Estradiol/ estron. All ovarian estrogens are produced from androgenic precursors. Estrogens are act on the CNS to induce behavioral estrus in the female; however, small amounts of progesterone with estrogen are needed to induce estrus in some species such as ewe and cow (Hafez and Hafez, 2000). With regard to the ovarian hormone feedback mechanism, estradiol-17β may play an important role in mediating so-called 'nutritional effects' in that its enhanced faecal excretion in well-fed ewes leads to reduced circulating plasma concentrations (Adam and Robinson, 1994), and an associated reduction in estradiol feedback that would be expected to enhance ovulation rate (Payne et al., 1991). Significantly higher concentrations of estradiol-17β were recorded on days 4 and 8 in insulin-treated goats compared to control ($P < 0.05$) and day 0 value ($P<0.01$) within same group (Sarath et al., 2008).

2.4.4 PROGESTERONE HORMONE.

Progesterone is a steroid hormone and the most prevalent, naturally occurring progestogen and is secreted by luteal cells of corpus luteum, the placenta, and adrenal gland. Progesterone is transported in blood by a binding globulin as for androgens and estrogens. LH primarily stimulates progesterone secretion. Progesterone prepares the endometrium for implantation and maintenance of pregnancy by increasing activity of secretory glands in the endometrium and by inhibiting the motility of the myometrium (Hafez and Hafez, 2000). The physiological role of progesterone in the regulation of the endocrine system as a circulating and a local regulator remains to be clarified. There was no

positive correlation between the number of the pulse frequency of follicle stimulating hormone (FSH) and the plasma concentrations of progesterone during the luteal phase in ewes (Bartlewski et al., 2000). Progesterone is the main hormone involved in the maintenance of pregnancy. Progesterone can be used to monitor the pregnancy status and timing of embryonic loss, as, in pregnant animals, progesterone concentrations remain elevated throughout gestation (Boscos et al., 2003). At present, the relationship between the effect of nutrition on jugular blood concentrations of progesterone and embryo mortality is not clear. It has been shown that there is an inverse relationship between peripheral progesterone concentrations and nutrition (Creed et al., 1994). This relationship is most likely a consequence of an increased catabolism of steroids by the larger livers often present in ewes fed a high energy diet (Parr et al., 1993). Titi et al., (2008) found that progesterone concentration increased ($P<0.05$) in ewes fed 3% fat addition but not for 5% level in Awassi sheep. While Titi and Awad (2007) observed that concentration of plasma progesterone were not affected by adding supplemental fat in goats. Significantly ($P < 0.05$) higher serum progesterone concentrations were recorded on days 12, 16, 20, and 24 in insulin- treated goats compared to control as well as day 0 value with in the same group. However, in controls it remained at basal level on almost all examination days (Sarath et al., 2008).

2.4.5 PROLACTIN HORMONE.

Prolactin is a polypeptide hormone secreted by the adenohypophysis. Prolactin initiates and maintains lactation. It is regarded as a gonadotropic hormone because of its luteotropic properties (maintenance of corpus luteum) in rodents. However, in domestic animals, LH is the main luteotropic hormone, with prolactin being of less importance in the luteotropic complex. Prolactin may mediate the seasonal and lactational effects on reproduction in farm animals (Hafez and Hafez, 2000).

2.5 THE ESTRUS CYCLE.

The estrus cycle is the recurring physiologic changes that are induced by reproductive hormones in most mammalian placental females. Reproduction in non-human mammals is regulated by an estrus cycle. In sheep, the length of the estrus cycle ranges

from 13 to 19 days and averages 17 days. The phases of the estrus cycle are proestrus, estrus, metestrus, and diestrus. Estrus is the period of time when the ewe is receptive to the ram and will stand for mating. It lasts approximately 24 to 36 hours. Ovulation (release of eggs by the ovary) occurs in mid to late-estrus. Metaestrus begins with the cessation of estrus and lasts for about 3 days. Primarily it is the period of the formation of corpus luteum (CL). The corpus luteum produces progesterone and maintains pregnancy in the ewe. Diestrus is the period of the estrus cycle when the CL is fully functional. Proestrus begins with the regression of the CL and drop in progesterone and extends to the start of estrus. Rapid follicular growth is occuring during this period. It usually extends from day 4 to day 13-15 of the cycle. Anestrus refers to a state where the normal cycle stops.

Estrus cycles are usually affected by the seasons. The number of hours daily that light enters the eye of the animal affects the brain, which governs the release of certain precursors and hormones. Most sheep are seasonally polyestrus and short-day breeders. They will begin to exhibit estrus when length of day begins decreasing. They will come into heat every 16 to 17 days until they are bred or return to anestrus. Thus, the most natural time for sheep to breed in the U.S. and Canada is the fall (Oct-Nov). Some sheep breeds are less seasonal. They breed almost year-round or have an extended breeding season. The less seasonal breeds include Dorset, Rambouillet, Merino, Finnsheep, Romanov, and hair sheep. The most seasonal breeds are the British long wool and meat breeds. The closer the flock is located to the equator, the longer the breeding season and the less complete and shorter will be the seasonal anestrus (Schoenian, 2005). High nutrition can also increase metabolic clearance rate of steroid hormones such as progesterone or estradiol. Lower concentrations of estradiol on the day of estrus are highly correlated with the occurrence of subestrus , there by making the detection of estrus in high yielding cows even more difficult (Roche, 2006). Whereas Suganuma et al., (2007) found that the progesterone has no physiological role in the regulation of FSH secretion and luteal function during the early luteal phase of the estrus cycle in goats.

2.5.1 SIGNS OF ESTRUS.

The signs of estrus in the ewe are much less pronounced than in the cow or doe and can usually not be detected unless a ram is present. When mature ewes are in heat, they will

seek out the ram and stand still for him to mount them. Sometimes they wag their tails vigorously. They may nuzzle the ram around the belly or scrotum and even try to mount the ram. Young ewes rarely exhibit these behaviors. There is evidence to suggest that rams and ewes prefer to mate with their own breed, but when there is no alternative ewes will mate with almost any breed of ram (Schoenian, 2005).

2.5.2 HORMONAL CONTROL OF THE ESTRUS CYCLE.

The estrus cycle is controlled through the balance of two hormones produced by the ovary, estrogen and progesterone. The egg develops within a structure called the follicle on the ovary. The cells surrounding the follicle produce estrogen. As the follicle grows and the egg develops, the level of estrogen produced increases. Estrogen is the predominant hormone for approximately 45 days in the estrus cycle. When the level of estrogen in the bloodstream reaches a high enough level, it activates an area of the brain that releases a hormone (a gonadotrophin) that causes the egg to ovulate. After ovulation the structure called the corpus luteum (CL) develops in place of the follicle and produces the second ovarian hormone, progesterone. Progesterone is the predominate hormone through most of the estrus cycle. It is also high throughout gestation in the ewe and acts to maintain the pregnancy. The corpus luteum regresses as the next egg develops. The fall in progesterone and the increase in estrogen will induce estrus behavior in the ewe (i.e. she will be receptive to the ram). Among other factors, a uterine hormone called prostaglandin causes regression of the CL. Administering external hormones is another means of inducing out of season breeding and synchronization of estrus in ewes problems with hormone release may result in a disruption of the cycle, which will decrease fertility. For instance, ovulation may not occur, which will cause the level of estrogen to remain high and halt the progress of the cycle. Alternatively, the corpus luteum may not regress which will cause progesterone to remain predominant. Postpartum anestrus in ruminants is an important cause of poor reproductive performance and economic losses for producers (Perez et al., 2002).

2.5.3 FACTORS AFFECTING ESTRUS AND OVULATION.

Although most breeds of sheep can carry and rear at least two lambs, lambing percentages are usually lower than 200%. Manipulating the ovulation rate when breeding in

or out of season, by pharmacological or natural methods, can improve lambing rates (Ptaszynska, 2006). De Fries et al., (1998) reported that fortifying ruminant animals with lipids might represent a practical means of affecting ovarian functions and promoting follicular development, hence leading to an increase in ovulation rate. Adding fat to a cow's diet may positively influence the reproductive performance of cows by promoting an earlier return to postpartum cycling activity (Espinoza et al., 1995).And supplementation with fat source resulted in increased follicular growth of beef (Bottger et al., 2002). Follicle stimulating (FSH) and luteinizing (LH) hormones are required for follicular growth, maturation and steriodogenesis in ovarian mammals. Postnatal development of ovaries and the variations in FSH and LH during this period of life are of great importance in determining reproductive capacity in all mammals. (Driancourt, 2001; Evans, 2003). Ferreria et al., (2008) found that the ovulation and LH concentration results suggest that the LH pattern observed in probably characteristic of the response of anovular Corriedale ewes to a sudden exposure to rams and estrus ewes.

2.5.3.1 RAM EFFECT.

This is a method of inducing estrus and ovulation in anestrus ewes during the end of the anestrus period season (Ptaszynska, 2006). The effectiveness of the male effect varies depending on a number of factors. Although establishing valid comparisons is difficult, response to the male depends on extent of breed seasonality (Ungerfeld, 2006). Ferreria et al., (2008) observed that anovular Corriedale ewes exposed to rams and estrus ewes during anestrus exhibited a pattern of LH secretion similar to that previously reported for Merino. The effect was more noticeable for mean LH concentration than for the number of LH pulses per hour.

2.5.3.2 GENETICS.

Breeds differ considerably in terms of ovulation rate, and crossbreeding is probably the simplest method of increasing the fecundity of a flock. On the other hand, there are individual animals, or strains of animals, in several breeds world-wide, which have a considerably higher ovulation rate than the mean for their flock or breed. The best-known examples are those Merino sheep carrying the Booroola or ‘F’ gene. Because this

characteristic lies in a single gene, it can be used, by back-crossing, to increase the ovulation rate substantially in any sheep population (Henderson and Robinson, 2000).

2.5.3.3 NUTRITION.

Ewes maintained on a low plane of nutrition usually have a low ovulation rate. It has been known for many years that a rising plane of nutrition, commonly known as 'flushing', may stimulate ovulation and increase litter size. However, the response to better quality feeding in the weeks prior to mating varies with the breed. Ewes generally respond best to flushing when in medium body condition rather than when excessively thin or fat (Henderson and Robinson, 2000). On the other hand, it has been demonstrated that low dietary intake can reduce ovulation rate in sheep and that dietary supplements containing high energy and protein can increase ovulation even in ewes in poor body condition and not being stimulated with exogenous gonadotrophins (Downing et al., 1995). O'Callaghan et al., (2000) found that non-stimulated ewes on a high quality dietary intake had a greater number of follicles compared with the ewes on a lower dietary intake. In general, in order to achieve reliable results, ewes should be allocated to groups, after weaning, depending on their condition score, and each group managed so that the majority is in the appropriate body condition prior to mating. In Australia, supplementation of the diet with lupin seeds has been found to improve ovulation rate. This effect appears to be independent of body condition and over-stimulation seems not to occur. Animals need to be fed lupin seeds at a rate of 500- 750 g/head/day for a minimum of 6 days before estrus, when a modest increase in ovulation rate of 20-30 ovulations per 100 ewes can be expected(Ptaszynska, 2006). Titi and Awad (2007) observed that using 5% supplemental fat adversely affected litter size and twining rate, but improved the kid birth weight in Shami goats. But De Santiago-Miramontes et al.,(2008) found the ovulation rate was not affected by nutritional supplementation in goats. While Butler et al., (2006) observed to the proportion of first postpartum dominant follicles that became ovulatory, atretic, or cystic was not different between control and propylene glycol cows. Nutritional effects on ovulation rate in ewes are not always accompanied by shifts in circulating FSH concentrations. Instead they may operate through altered ovarian hormone feedback mechanisms that influence the duration of exposure of the gonadotrophin-dependent follicles to FSH or through threshold-reducing

actions of specific nutrients and dietary-induced metabolites on the amount of FSH needed to support the gonadotrophin- dependent follicles. Recent results suggest that the enhanced faecal excretion of estradiol by well-fed ewes may increase ovulation rate by reducing ovarian estradiol feedback (Robinson, 1996). Energy supplementation with forage seems to be as effective as energy supplementation with concentrate for enhancing reproductive efficiency after estrus synchronization treatment in thin cows. An increase in energy level, well known for improving the fertility rate at induced estrus, seems to be more important than the quality of energy supply. Energy supply with hay or grass silage might have different effects on reproduction. Nevertheless, cheap planes of nutrition based on maize silage may be applied in herds when concentrate availability is low (Ponsart et al., 2000). The effect of a short-term nutritional supplementation on ovulation rate in the ewes may be mediated through an increase in the concentration of glucose, insulin and leptin (Gil, 2003). Nutritional supplementation before parturition assured good body condition at calving and suggested that it was effective at increasing cholesterol availability to maintain ovarian follicle function and to favor earlier resumption of ovarian activity (Filho et al., 2010).

2.5.3.4 GONADOTROPHINS.

Gonadotrophin-releasing hormone (GnRH) regulates the synthesis and release of the gonadotropic hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from gonadotrope cells of the anterior pituitary (Wilson et al., 1990). However, dissociation in the pattern of FSH and LH secretion occurs during the estrus cycle of cattle (Sunderland et al., 1994). Recurrent increases in FSH concentrations occur throughout the estrus cycle, associated with emergence of follicular waves (Sunderland et al., 1994; Crowe et al., 1997). Gonadotrophins, such as PMSG or porcine follicle stimulating hormone (pFSH), can be used to superovulate ewes (Henderson and Robertson, 2000). These treatments need to be administered to cyclic ewes during the follicular phase of the estrus cycle, or after a period of progesterone priming when used outside the breeding season. The pituitary-derived gonadotrophins (e.g. pFSH) are short-acting and require frequent injections, so their use is restricted, in practice, to embryo transfer programs (Heresign, 1992). PMSG is longer lasting and usually used for inducing estrus and ovulation outside the normal breeding season or for ensuring good conception rates at a synchronized estrus

in a fixed timed insemination programe during the breeding season (Husein et al., 1998; Henderson and Robertson, 2000). Gonadotrophins are a heterogeneous population of glycoproteins whether in circulation or within the pituitary gland of sheep (Arrieta et al., 2006). The distribution and proportion of gonadotrophin isoform in the eluent do not change in ovariectomized heifer's pituitary gland after treatment with progesterone. And there are other intra-ovarian factors along with progesterone are involved in regulation of gonadotropin isoform distribution in the pituitary gland of cattle during the luteal phase of the estrus cycle (Perera-Marin et al., 2008). Ewes in a high body condition had a higher ovulation rate, which was associated with higher FSH and lower estradiol concentration during the follicular phase of the cycle (Gil, 2003).

2.6 NATURAL MATING.

In natural mating conditions, the length of the estrus cycle and the duration of estrus mean that about 6-8% of ewes will be in estrus each day of the breeding season. Assuming there is a ram for every 50 ewes (50:1 ratio) each will need to mate an average of 3-4 ewes per day. This is compatible with the serving capacity of the ram and allows for good fertility. The high concentration of spermatozoa per ejaculate, together with the repeated mating of the ewe throughout estrus, ensures a good level of fertility and prolificacy. However, the reproductive performance of rams is affected by seasonal influences (Henderson and Robinson, 2000) and the requirements of out-of-season breeding and the greater number of ewes coming into estrus as a result of synchronization impose the need for a more rational use of rams. Fertility increases as estrus progresses, reaching a maximum towards the end of the estrus period. Therefore, the only way to increase fertility, while at the same time optimizing the use of the ram, is to practice 'hand mating'. This involves the rams being lined up in a queue in the shedding race and each ram in turn being exposed to a group of (preferably synchronized) ewes. Following an observed mating, the ewe is withdrawn from the group and the ram is taken to the back of the queue. The next ram in line is then exposed to the unmated ewes. The improvement of desirable production traits requires the selection of superior animals for breeding. Since rams are responsible for more offspring than ewes, ram selection is critical. One of the ways of managing selective breeding is batch mating; a group of ewes is mated exclusively by the same ram, using

'hand mating' after estrus detection or synchronization, or by artificial insemination (Ptaszynska, 2006).

2.7 FERTILITY.

Fertility, the proportion of ewes lambing of all those exposed to the ram during a defined period (usually expressed as a percentage) varies with breed, season, age, nutritional status, breeding management and farm conditions. An average figure of 70 to 80% following natural mating is considered normal to good for autumn breeding, and good to very good for spring breeding. Artificial insemination (AI) produces poorer results than these (Ptaszynska, 2006). Hoedemaker et al., (2004) found measures of fertility did not differ between the control group and propylene glycol group supplementation in dairy cows. Infertility is one of the most important economic losses in high producing dairy cows. Though these losses are largely attributed to health related factors such as retain placenta, metritis, anestrus, silent estrus, cystic follicles, repeat breeding and abortions/still birth, poor feeding and management have often reportedly predisposed the cows to infertility causing factors (Lanyasunya et al., 2005). The nutrition of early lactation cows has an important influence on their fertility performance. Negative energy balance caused by inadequate nutrient supply or excessive consumption is able to affect the fertility of female mammals. In particular, excessive loss of body weight during lactation in cattle and swine accentuates the decrease in energy balance and is able to extend the interval from parturition or weaning to estrus, respectively (Mattos et al., 2000).

In particular, the energy and protein balance of early lactation dairy cows are factors, which are likely to be implicated in poor fertility for Irish herds quite often (Muligan et al., 2007). It is now widely recognised that reduced fertility in dairy herds is one of the most important factors affecting producer profitability. Roche, (2006) has defined the effect of infertility on profitability as: (i) prolonged calving interval with fewer calves and less milk per cow; (ii) increased replacement costs; (iii) increased labour, semen and veterinary bills; and (iv) an extended low production or dry period which increases body condition score (BCS) at calving and also reduces fertility at the subsequent breeding season.

2.8 PARTURITION (LAMBING).

There are three stages to parturition (lambing): 1) dilation of the cervix; 2) expulsion of the fetus; and 3) expulsion of the placenta. Stage one usually takes 3 to 4 hours. The birth of a lamb usually occurs within an hour of less from the rupture of the first water bag. A ewe lambing for the first time or with multiple births may take longer. If labor takes over an hour for mature ewes and over 2 hours for ewe lambs, assistance may be required. The placenta is passed 2 to 3 hours after delivery is finished. In multiple births, there are separate afterbirths for each lamb. After the lamb is born, the ewe will lick and nuzzle it to begin the bonding process (Schoenian, 2005). Titi and Awad, (2007) found that the gestation length was increased ($P<0.05$) by feeding supplemental fat in goat. Taylor et al., (2009) observed that ewes lost weight while grazing winter range, but mature body weight, once achieved, was restored annually, with the exception of the 7-yr-old Columbia and Targhee ewes. Regardless of breed or age, ewes were able to achieve lambing rates >1.5 lambs after early- to mid-pregnancy weight loss. Lambing rates were greater in older ewes, which generally lost the greatest amount of body weight during winter grazing, except for 7-yr-old Targhee ewes.

2.9 BIOTECHNOLOGY IN SHEEP REPRODUCTION.

In the last 10-20 years, new biotechnologies have been developed that are on the verge of revolutionizing reproductive processes in humans and animals. In agriculture, modern techniques in assisted reproductive technology (ART) are being used for the introduction, improvement, and preservation of livestock genetics and the enhancement of animal reproductive efficiency. Modern embryology and ART technologies have facilitated the development of methods to transfer desired single genes or, alternatively, the entire genome from desirable individuals or embryos. In addition, rapid advances in techniques to manipulate embryos in the laboratory have permitted screening of embryos for genetic defects or highly desirable quantitative traits using molecular markers. The ART techniques include artificial insemination, estrus synchronization, estrus induction, synchronization of parturition, superovulation, in vitro fertilization, in vivo and in vitro embryo production, embryo collection, embryo transfer, embryo cryopreservation, embryo splitting, cloning, production of transgenic animals, and preimplantation genetic diagnosis. The sheep is an

excellent model for all listed techniques and has been used extensively in basic and applied research (Grazul-Bilska, 2004).

2.10 NUTRITION AND REPRODUCTION.

Nutrition plays a key role in regulating the reproductive performance in farm animals. Restriction of energy intake has a major role in increasing the length of postpartum anestrus in sheep and cattle (Schillo, 1992). The relationship between nutrition and reproduction in ruminants is complex and responses are often variable and inconsistent. In sheep, low dietary intake can reduce ovulation rate and dietary supplements containing high energy and protein can increase ovulation rate in ewes with poor body condition that are not supplemented with exogenous gonadotrophins (Downing et al., 1995).

Reproductive efficiency of sheep flocks is the product of three factors: fertility, prolificacy and the lambs' survival. Prolificacy, which is determined by ovulation rate, is a key factor in reproductive efficiency that can be improved by nutrition (Scaramuzzi, 1988). The relationship between nutrition and reproduction is a topic of increasing importance among dairy producers, veterinarians, feed dealers and animal nutrition experts. This is due to the recognition of the fact that high reproductive efficiency in dairy herds is dependent upon good nutrition and management (Lanyasunya et al., 2005).

Nutrition has a direct bearing upon reproductive performance. Ewes kept in acceptable condition before breeding normally produce more lambs if they are flushed, or given the chance to gain weight before and during the breeding season. They can be flushed with rested pastures or by supplementation. Begin flushing three weeks before breeding and, if possible, continue through the first cycle (approximately 17 days).Flushing ewes is most effective when they are mated early in the breeding season. Since ovulation rate is near a maximum during the middle of the season, flushing at this time is not as beneficial. The results of flushing are quite variable. Sometimes, when farm flock ewes are already on a high nutrition level before the breeding season, flushing may not affect ovulation or lambing percentage. Nutrition affects total lifetime productivity of sheep by influencing mature size. Well-developed ewes consistently have higher lamb crop percentages than smaller ewes. Fat ewes, however, are typically less fertile, do not respond to flushing, and may experience more embryonic death loss. Ewes grazed on legume pastures, such as

alfalfa and clover, may at times be less fertile. Under some conditions, the estrogen content of these legumes is related to reproductive disorders. Breeding dates may be delayed and conception rate reduced when ewes are on pastures that have high estrogen content. However, the estrogen content of legumes declines during the later stages of maturity. Underfed ewes showed a significant decrease ($P< 0.001$) in body weight, whereas ewes fed the ad libitum diet showed a significant increase ($P< 0.001$) in body weight during the period in which the nutritional treatments were applied (Lozano et al., 2003). Nutritional status of the ewe during pregnancy is critical, as it has major implications through its effect on ewe reproductive efficiency and colostrum production, and also on fetal development and subsequent lamb survival and performance. Nutrition influences certain metabolic pathways, not only directly by providing essential nutrients, but also indirectly by modifying the expression of hormonal functions, which in turn influence oocyte maturation, ovulation, embryo development, fetal growth and the viability and vigour of the newborn lamb (Robinson et al., 2002). Ewe changes undergone to compensate for nutrient shortage or allowance during early to mid-pregnancy have the potential to alter the performance of the offspring. A low plane of nutrition in early pregnancy was associated with lambs with improved immune status, higher growth rates and reduced mortality to weaning. A medium plane of nutrition during mid-pregnancy was associated with increased fetal growth and a larger skeletal size at birth. (Munoz et al., 2008). While Demirel et al., (2004) observed that supplementary feeding in addition to grazing during breeding season increased live weight in Norduz ewes, this practice did not effect twinning rate, lamb number per mated and lambed ewes, lamb weights at various ages and survivability rates. Maternal under nutrition and, under certain circumstances overnutrition, before or during pregnancy or during early postnatal life can alter reproductive function of the offspring. Effects can be exerted at many stages of development, from prior to conception until after birth and may be expressed at the time of the nutritional insult or later (Rhind, 2004). Lassoued et al., (2004) showed that for the low prolific Queue Fine de l'Ouest breed ewes, increasing the level of nutrition through a heavier use of concentrate did not improve ovulation rate but substantially depressed litter size as a result of higher reproductive losses. The ewe's reproductive response and the results obtained on fetal development and offspring performance following nutritional manipulation during early and mid-pregnancy

are influenced by critical factors such as maturity of the ewe, level and length of feeding regime, and stage of pregnancy (Redmer et al., 2004).

2.10.1 PASTURE.

Permanent pasture should be the predominant source of nutrition for the sheep flock. Intensive sheep production systems where the sheep are housed and fed harvested feeds are not as profitable as more extensive production systems where they harvest their own feed. When a sufficient quantity of forage is available, sheep are able to meet their nutrient requirements from forage alone along with a supplemental source of salt and minerals. Clover should be over seeded on permanent pastures in the winter to improve the quantity and quality of forage produced during the grazing season. Sheep prefer to graze leafy, vegetative growth that is 2 to 6 inches tall rather than steamy, more mature forages. Pasture growth is not distributed evenly throughout the year. Approximately 60 percent of the annual dry matter production of most species of cool season grasses occurs in the spring. When pastures are not stocked heavily enough to utilize the spring flush of growth, sheep graze and regress certain areas while other areas are left to mature and go to seed. This type of grazing behavior weakens those plants that are grazed more frequently and gives the less desirable plants a competitive advantage. Approximately one-third of spring pasture should be fenced for hay production. After hay cutting, pasture should be given a three- to four-week recovery period before making it available for grazing the remainder of the year. Rotational grazing programs designed for the movement of sheep every 10 to 14 days are instituted in late June and early July to improve both pasture and lamb production. More intensive rotational grazing systems where higher stocking rates are used help to promote more complete forage utilization, but also require greater input costs in the form of fence and water and may result in higher levels of internal parasitism, increased risk of coccidiosis, and impaired lamb performance. Pitta et al., (2004) indicated that grazing of willow fodder blocks during mating has proved to be beneficial for increasing reproductive rate in ewes. Moore et al., (2003) found that willow supplementation of beef cattle grazing dry summer pastures reduced live weight loss under prolonged summer drought conditions. McWilliam et al., (2003) established that supplementing ewes with poplar and willow cuttings when grazing drought pasture during mating increased reproductive rate and

reduced live weight loss. Supplementing willows to sheep and cattle proved to be effective but demanded large labour costs. Aguilar-Perez et al., (2009) indicated that supplementation with cereal-based concentrates in early lactation can reduce or eliminate negative energy balance in crossbred cows under grazing conditions in the tropics. Furthermore, supplementation with cereal-based concentrates can improve milk yield and tends to improve reproductive parameters. Together with the results of our previous study, the findings suggest that cereal based supplements are likely to be more beneficial than fatty acid supplements for overcoming the constraints of low grass quality and availability for dual-purpose cattle in the tropics. Carrasco et al., (2009) observed that grazing lambs presented lower dressing percentage, fatness degree and total body fat depots, being the subcutaneous fat the most reduced fat depot. The supplementation with concentrate in grazing lambs improved the animal performance and carcass yield. Feeding system was not associated with any important changes in subjective meat and fat colour, fat consistence or joint proportions.

2.10.2 PROTEIN.

In sheep rations, the amount of protein is much more important than quality of protein. However, since the sheep is a ruminant, mature sheep use effectively the naturally occurring protein and nonprotein nitrogen (urea) in their diets. Common sources of natural protein supplements include cottonseed, soybean, sunflower, linseed, and peanut meals. These oilseed meals contain from 40 to 50 percent protein and are excellent sources of supplemental protein. High-quality legume hays can contain from 12 to 20 percent protein and provide adequate protein for most classes of sheep when fed as a complete ration. Grains, however, are low in protein. They generally contain only 8 to 11 percent protein. Additional protein is necessary in high-grain, lamb-finishing rations for maximum performance.

Nonprotein nitrogen sources should not be fed to young lambs. Young lambs are not functioning ruminants until they are approximately 2 months old, depending upon how soon they have access to grain and forage. However, mature sheep can be fed low levels of nonprotein nitrogen. In general, supplemental nonprotein nitrogen is beneficial only when adequate energy is available. Urea should never make up more than one-third of the

ruminally degradable protein in the diet. Additionally, nonprotein nitrogen sources should not be used when lambs are limit-fed. Urea can be toxic if consumed in large amounts over a short time, especially when the diet lacks ruminally available energy. Furthermore, urea is very unpalatable. When supplementing range sheep in New Mexico, it is important to consider the quantity of available forage in the pasture. If adequate forage is present, but the standing forage is dry and brown (containing < 5 to 6 percent crude proteins), it may be necessary to supplement with a high-protein feed (> 35 percent protein). However, if the amount of available forage is insufficient or if the forage is still somewhat green (> 6 percent protein), a lower-protein supplement should be fed to provide additional energy, if needed. Lactating ewes have the highest protein requirement and may require supplemental protein if the range forage contains less than 10 to 12 percent crude protein. El-Sherif and Assad (2001) found that plasma protein with its two components, albumin and globulin, increased significantly at the 6^{th} week, but dropped throughout the 16-18^{th} week of pregnancy. But urea began to increase significantly after 10-12 weeks of pregnancy in Barki ewes.

2.10.3 MINERALS.

Approximately 13 different minerals are essential in sheep nutrition. Most of these requirements are met under normal grazing and feeding habits in New Mexico. Those that are most deficient are salt (sodium chloride) and phosphorus. Salt is essential for many body functions. When sheep are deprived of salt, they generally consume less feed and water, produce less milk, and grow slowly. Animals that are deprived of adequate salt may try to satisfy their needs by chewing wood, licking dirt, or eating toxic amounts of poisonous plants. Inadequate salt intake may cause decreased feed consumption and decreased efficiency of nutrient use. When adding salt to mixed feed, add 0.3 percent to the complete diet or 1 percent to the concentrate portion. In general, supplemental salt should be provided to range ewes at a level of 8 to 11 g of salt per head per day. Provide loose salt rather than salt blocks. Sheep tend to bite instead of lick salt blocks; as a consequence, their teeth may break or wear down prematurely. Almost all pastures and hays contain an abundance of calcium, but grains are lower in calcium. When lambs are fed on a high-concentrate diet, calcium supplementation may be necessary. Trace element deficiency may

be linked to problems such as retained fetal membranes (Gupta et al., 2005), abortion (Mee, 2004) and weak calf syndrome (Logan et al., 1990). Furthermore, Husband (2006) has recently reported combined selenium and iodine deficiency in a dairy herd with a high incidence of retained fetal membranes, milk fever and vulval discharge. In other cases, differences in the reproductive performance of cattle and sheep have been reported when comparing trace element supplementation strategies (Hemmingway, 2003; Black and French, 2004). The trace element; cobalt is a dietary essential element for ruminants, allowing synthesis of vit. B_{12} by rumen microorganisms (Tiffany et al., 2003). Cenesiz et al., (2006) found that lambs feed supplemented with urea molasses mineral blocks (UMMB) increases microbial activity and fermentation of rumen, and there is also an increase in nitrogen and minerals supplies that are received by organism. Bademkiran et al., (2008) observed that if cows are fed additional trace elements during certain periods, it may be possible to prevent disorders caused by deficiencies of those elements in cattle kept tied indoors. A shortage of these elements may cause a decline on the total performance of cattle and consequently economic loss. Copper is essential for the normal functioning of all living organisms as a crucial cofactor for enzymes involved in the development of nervous system, respiration and iron metabolism. Copper also plays an important role in female reproduction (Michaluk and Kochman, 2007). The copper supplemented at 40 mg/kg of DM to lactating dairy cattle diets can increase liver copper concentrations to levels indicative of copper toxicity, but clinical signs of copper toxicity may only be manifested over a prolonged duration of copper supplementation. Contrary to results reported with steers, copper supplementation to dairy cattle adequate in copper (based on plasma and liver copper concentrations), resulted in an increase in serum cholesterol levels. This may have ramifications related to the improvement in reproductive performance of dairy cattle (Engle et al., 2001).

2.10.4 VITAMINS.

Mature sheep require all the fat-soluble vitamins: A, D, E, and K. They do not require supplemental B vitamins, which are synthesized in the rumen. Normally, the forage and feed supply contain all essential vitamins in adequate amounts, except vitamin A, which is sometimes deficient in dormant forage. However, sheep can store vitamin A for a

considerable time. If ewes have been pastured on green forage or have had access to high-quality legume hay, vitamin A is not usually deficient.

In some areas, lambs may develop white muscle disease. This is thought to be caused from a deficiency of vitamin E, selenium, or both. Treatment is most effective with early diagnosis and injection of a vitamin E-selenium material.

2.10.5 ENERGY.

Energy is the most common nutritional deficiency for ewes. Forages provide the primary, and in some cases, the sole source of energy. At production stages when energy requirements are increased (flushing, late gestation, lactation), you can supplement energy with concentrate feeds such as barley, wheat, oats, and corn. An energy deficiency can reduce conception rate, reduce lambing rate and milk production. It may also negatively affect wool production. Energy deficiency is linked to greater susceptibility to parasite infestation and is also the primary cause of pregnancy toxemia (ketosis) in late pregnancy. Energy deficiency reduces rate of gain in growing animals. In severe cases, it causes weight loss or even death. Excess energy intake also can reduce productivity. Over-conditioned (fat) ewes are reproductively less efficient and have more lambing difficulties. Excess energy intake is most likely to occur in ewes grazing highly productive pastures after weaning their lambs. It is well documented that cows bred while losing body weight and body condition have decreased pregnancy rates. Data from the present study indicate dietary energy restriction affects follicular growth and corpus luteum development in nonlactating beef cows. This abnormal corpus luteum development may be a cause for decreased pregnancy rates. However, not all nonlactating cows fed diets limited in energy develop subfunctional corpora lutea before becoming anestrus (Burns et al., 1997). Although dietary fat is often added to the diets of ruminants to increase energy intake, an additional response to fat supplementation has improved fertility (Staples et al., 1998). In dairy cattle, both the duration and severity of early postpartum negative energy balance (NEB) are correlated with the interval to resumption of ovulatory activity following parturition. The principal defect caused by NEB occurs at the level of the hypothalamus, manifested by reduced GnRH pulse frequency. The NEB results in a parallel reduction in pulsatile pituitary LH release, with consequent compromised follicular steroid output and

anovulation. In addition, follicular responsiveness to gonadotropin stimulation is blunted by the circulating hormonal and metabolite environment of the NEB state. Moreover, hypothalamic responsiveness to the negative feedback effects of estradiol is enhanced, resulting in further attenuation of already suboptimal GnRH pulse frequency (Beam and Butler, 1999; Wiltbank et al., 2002; Diskin et al., 2003). Cenesiz et al., (2006) found that lambs feed supplemented with urea molasses mineral blocks (UMMB) increases microbial activity and fermentation of rumen, and there is also an increase in nitrogen and energy supplies that are received by organism.

2.10.5.1. FEED ENERGY.

Insufficient energy limits performance of sheep probably more than any other nutritional deficiency. An energy deficiency may result from inadequate amounts of feed or from feeds (generally forages) that do not contain enough protein to sufficiently "unlock" the energy in the feedstuff. The major sources of energy for sheep are hay, pasture, silage, and grains. Milo, barley, corn, oats, and wheat also can be used to raise the energy level of the diet when necessary. Energy deficiencies can cause reduced growth rate, loss of weight, reduced fertility, lowered milk production, and reduced wool quantity and quality. Energy level has been related to variations in LH secretion and follicular development (Lucy et al., 1992; Grimard et al., 1995). Khireddine et al., (1998) showed that energy supplementation (2 kg concentrate+15.0 MJ Net Energy/day) from 11 days before estrus synchronization treatment to 3 weeks after artificial insemination enhanced reproductive efficiency in postpartum feed-restricted (70% of energy and protein requirements) beef cows. To reduce production costs, the same amount of additional energy could be given using a different form of supplementation. There are many different reports which indicate that the type of energy fed has a significant influence on dairy cow fertility. It has been reported that feeding diets which result in a relatively high supply of glucogenic nutrients (mostly ruminal propionate and glucose) results in less mobilization of adipose tissue as measured by blood metabolites (Rizos et al., 2004). In addition, there are some reports that such diets improve fertility in dairy cattle (Gong et al., 2002).

Cholesterol is a lipidic, waxy alcohol found in the cell membranes and transported in the blood plasma of all animals. It is an essential component of mammalian cell membranes

where it is required to establish proper membrane permeability and fluidity. Cholesterol is the principal sterol synthesized by animals, but small quantities are synthesized in other eukaryotes. Cholesterol is classified as a sterol (a contraction of steroid and alcohol). It is the main precursor of vitamin D and of the steroid hormones, which include cortisol and aldosterone (in the adrenal glands) and progesterone, estrogens, and testosterone (the sex hormones), and their derivatives. It provides the basic structure of all the steroids. During the prepartum period the ewes fed propylene glycol showed lower plasma total cholesterol than ewes fed control diet (Chiofalo et al., 2005). Balikci et al., (2007) found that changes occur in the blood metabolite concentrations, especially at 100 and 150 days of pregnancy. The levels of blood cholesterol and triglyceride increased. The magnitude of these changes was greater in twin-bearing sheep which could be attributed to increased nutrient demands of the fetuses.

Triglyceride is glyceride in which the glycerol is esterified with three fatty acids. It is the main constituent of vegetable oil and animal fats. Triglycerides, as major components of very low density lipoprotein, play an important role in metabolism as energy sources and transporters of dietary fat. Fats in the diet can influence reproduction positively by altering both ovarian follicle and corpus luteum function via improved energy status and by increasing precursors for the synthesis of reproductive hormones such as steroids and prostaglandins (Mattos et al., 2000). Fish oil (FO) contains relatively high concentrations of two polyunsaturated fatty acids of the n-3 family: eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6). These fatty acids can be supplied only by the diet because EPA and DHA cannot be synthesized de novo in mammalian systems. Eicosapentaenoic acid and DHA have inhibited secretion of PGF2α in different animal cell culture systems (Achard et al., 1997), including bovine endometrial cells (Mattos et al., 2001). Chiofalo et al., (2005) observed that propylene glycol had no effect on plasma triglyceride in dairy ewes. The central role of the liver in coordinating metabolism and reproduction is illustrated by the negative correlation between liver triglycerides and days to first estrus and to pregnancy (Jorritsma et al., 2000).

2.11 GLUCOPLASTIC SUBSTANCES AND OLEOCHEMICAL PRODUCTS IN NUTRITION.

Propylene glycol is a glucogenic precursor that is either rapidly absorbed from the rumen and converted to glucose, or partially metabolized to propionate in the rumen before being absorbed (Nielsen and Ingvartsen, 2004).

Oleochemicals are chemicals derived from plant and animal fats. They are analogous to petrochemicals which are chemicals derived from petroleum. The formation of basic oleochemical substances like fatty acids, fatty acid methyl esters, fatty alcohols, fatty amines and glycerols are by various chemical and enzymatic reactions. Glycerol as a glucoplastic substance is used to prevent nutritional deficiencies in dairy cows predominantly during the transition period which encompasses the last 3 weeks prepartum and first 3 weeks postpartum (Goff and Horst, 2001). Glycerol as a glucoplastic substance is involved in the metabolism of glucose. This natural product can be found in all vegetable and animal fats. Its incorporation in the glycide metabolism is more expeditious than that of propylene glycol and other chemical glucoplastic substances (Bartelt and Schneider, 2002).

2.11.1 GLYCOLS.

Glycol is a chemical compound containing two hydroxyl groups (-OH groups). Vicinal diols have hydroxyl groups attached to adjacent atoms. Examples of vicinal diol compounds are ethylene glycol and propylene glycol. Propylene glycol, known also by the systematic name propane-1, 2-diol, is an organic compound (a diol alcohol), usually a faintly sweet, odorless, and colorless clear viscous liquid that is hygroscopic and miscible with water, acetone, and chloroform. Propylene glycol can also be converted from glycerol, a biodiesel byproduct. Propylene glycol is a glucoplastic substance for ruminants, which has been used in the treatment of ketosis since the 1950s and is still used today. It may be used to reduce the negative energy balance after calving and limiting the risk of ketosis and fatty liver. Propylene glycol may affect glucogenic action in different ways. The portion of this substance is metabolised in the rumen to lactic acid and propionic acid, which are converted to glucose by hepatocytes; the propylene glycol, which escapes rumen fermentation, is absorbed by the rumen wall or from the gastrointestinal tract and is converted to glucose by the liver (Cozzi et al., 1996). Chiofalo et al., (2005) found that

feeding propylene glycol to dairy ewes from 30 days prepartum up to 30 days postpartum increased glucose concentration in plasma and milk yield. Rekik et al., (2007) observed that the proportion of ewes detected in estrus was higher in the ewes fed with polyethylene glycol than in the ewes fed without polyethylene glycol. The glucogenic substance propylene glycol (PG) has been shown to decrease the negative effect of decreased Dry matter intake (DMI) on negative energy balance (Studer et al., 1993; Formigoni et al., 1996) and to lower the risk of ketosis and fatty liver syndrome (Studer et al., 1993). However, little is known about the effects of propylene glycol supplementation in the transition period on health, fertility, and milk production, which are important factors to evaluate the profitability of this measurement. Castaneda-Gutierrez et al., (2009) found the net energy balance during the peripartum period tended to be greater in cows fed propylene glycol compared with other groups.

2.11.2 GLYCEROL.

Glycerol is a chemical compound also commonly called glycerin or glycerine. It is a colorless, odorless, viscous liquid that is widely used in pharmaceutical formulations. for human consumption, glycerol is classified by the FDA among the sugar alcohols as a caloric macronutrient. Glycerol has three hydrophilic hydroxyl groups that are responsible for its solubility in water and its hygroscopic nature. The glycerol substructure is a central component of many lipids. Glycerol is sweet-tasting and of low toxicity, and it is a 10% by-product of biodiesel production (via the transesterification of vegetable oils or animal fats). This has led to an excess of crude glycerol in the market, making the epichlorohydrin process no longer economical. Glycerol is also used as a sugar substitute. In this regard, it has approximately 27 calories per teaspoon and is 60% as sweet as sucrose. Although it has about the same food energy as table sugar, it does not raise blood sugar levels, nor does it feed the bacteria that form plaques and cause dental cavities. As a food additive, glycerol is also known as E number E422.

The term ‘bio-diesel’ is used to describe the methyl or sometimes ethyl esters produced from oilseed crops. Every 10 gallons of biodiesel produced generates about 7.6 lb of crude glycerol. According to the National Biodiesel Board, the production of biodiesel in the U.S. over the next decade is expected to grow. Biodiesel is an alternative automotive fuel that

can be produced from vegetable oils and (or) animal fats. In general, the oil or fat is mixed with an alcohol, usually methanol, and a catalyst (often sodium hydroxide) that causes triglycerides to separate, forming methyl esters (biodiesel) and crude glycerol. Crude glycerol is the principal co-product of biodiesel production (Thompson and He, 2006) and has been proposed as a potential beneficial energy source for pigs (Lammers et al., 2008a) and poultry (Dozier et al., 2008; Lammers et al., 2008b). Key factors, however, determining the nutritional value of crude glycerol for pigs are the feedstock source and the manufacturing process (Thompson and He, 2006). Glycerin is generally recognized as safe for use in animal feed (FDA, 2006).

As early as the 1950s, glycerol was used to treat ketosis in dairy cows via drenching orally, feeding with concentrates, or both, with a relatively large dose (Johnson, 1954). At that time, however, the cost of glycerol made it less economically feasible as a treatment for ketosis compared with propylene glycol. More recently, surplus production of glycerin (or glycerol) from biodiesel fuel production has made various forms of glycerin an attractive glucogenic substrate for feeding livestock. Surplus glycerin from biodiesel fuel production will likely flood glycerin supplies for the traditional uses, although there are many applications for glycerin, such as using it as an energy source in livestock diets. From very limited research, glycerin has been fed as a feed ingredient to replace energy sources (Chung et al., 2007).

Glycerol of different purities can be included in mixed diets for ruminants up to 10% of the dry matter as a substitute for rapidly fermentable starch sources, e. g., wheat or tapioca, without negatively affecting ruminal environment, ruminal nutrient turnover and whole-tract digestibilities of organic matter constituents. Mean energy concentrations of glycerol derived from diets fed to sheep and containing a low-starch or a high-starch concentrate were 9.7 and 8.3 MJ NEL/kg of glycerol, respectively. When fed with a low-starch concentrate, pure glycerol at dietary inclusion levels up to 20% had no effect or positive effects on nutrient digestibilities. When included in diets containing high-starch concentrates, glycerol reduced cell-wall digestibilities but had no obvious effect on whole-tract organic matter digestibilities. Chemical as well as physical pellet quality variables were not affected greatly. The preserving effect on concentrate pellets of the glucogenic substance glycerol must be emphasized. The results of these studies suggest that the

glucose precursor glycerol is an excellent feed constituent, even when included in an impure form as derived from biodiesel production. Glycerol may serve as an ingredient both of pelleted concentrates and of total mixed rations. In pelleted concentrates, the contribution to the hygienic quality of the feedstuff might be of special interest. Economic assessment will be decisive of a wider use of glycerol as a dietary ingredient for ruminants. The crude glycerol could be included in finishing pig diets without adversely affecting the growth performance or meat quality of pigs. However, blood glycerol levels became elevated after prolonged feeding that may reduce the efficiency of glycerol when used as an energy source for pigs. Furthermore, diets that contained more than 8% glycerol formed afirmaggregate within 24 h of mixing that presented some feeding difficulties, which most likely limits crude glycerol to dietary levels less than this in mash diets. Crude glycerol may play a role in the pig industry by supplying energy at a more cost effective price than competing energy ingredients; however, a shadow-pricing exercise is necessary to ascertain whether glycerol can be economically included in current diets (Hansen et al., 2009).

Studies examining the effects of supplementing crude glycerol to diets fed to swine (Mourot et al., 1994; Kijora and Kupsch, 2006) and broilers (Simon et al., 1996; Cerrate et al., 2006) have shown little to no effect on animal performance. Schroder and Sudekum (1999) fed 10% glycerol to dairy cattle, effectively replacing over one-half of the starch in the diet, without negatively affecting intake, ruminal digestibility, rumen microbial synthesis, or total tract nutrient digestibility in steers. Feeding 3.6% glycerol to mid-lactation dairy cows was without effect on intake, milk production, or gross milk composition but slightly altered the profile of fatty acids in milk and increased rumen propionate and butyrate concentrations at the expense of reduced acetate concentration (Khalili et al., 1997). Feeding 1.89 lb/day of glycerol to +21 days relative to calving (5.4% of ration DM) did not have any effects on milk production or feed intake (DeFrain et al., 2004). Feeding 500 ml of glycerol, or approximately 3.1% of ration DM, from 3 weeks prior to calving through 70 days in milk caused an increase milk yield and milk protein content (Bodarski et al., 2005). Because glycerol has not been used as a macro ingredient, the estimates of net energy of lactation (NEL) are not available for typical feeding scenarios. Schroder and Sudekum (1999) reported estimates from 0.9 to 1.03 Mcal/lb with energy values decreasing for higher starch diets, and recently, DeFrain et al., (2004)

reported 0.86 Mcal/ lb when feeding glycerol in early lactation. There is uncertainty in the energy value for glycerol due to the amounts fed previously and unknown interactions with other ration components.

Glycerol is fermented to volatile fatty acids (VFA) in the rumen. Glycerol fermentation using rumen fluid inoculum from cows adapted to glycerol feeding indicates increased production of propionate and butyrate at the expense of acetate (Remond et al., 1993). Glycerol is a valuable feed ingredient for lactating dairy cows. Glycerol can be included as a macro ingredient in diets for lactating dairy cows without any deleterious effects. Therefore, feeding glycerol in place of corn is an alternative strategy for formulating diets for lactating cows when corn is not priced favorability (Donkin and Doane, 2007). Serum insulin concentration ($P < 0.05$), and insulin to glucose ratio ($P<0.05$) were observed in bulls fed 8% glycerin in concentrate compared with those receiving 0, 4, or 12%. No changes were observed in carcass and meat quality. In addition, feeding concentrate containing up to 12.1% of glycerin does not lead to detrimental effects on performance, ruminal fermentation, metabolism, and carcass and meat quality variables (Mach et al., 2009). Wang et al., (2009a) found that Increasing supplementation of glycerol in the diet of steers linearly increased rumen volatile fatty acid concentration and altered rumen fermentation pattern into more propionate production.

Glycerol has been fed to dairy cows in early lactation (DeFrain et al., 2004; Bodarski et al., 2005; Ogborn, 2006) or cows in mid lactation (Khalili et al., 1997) as an energy supplement. Bodarski et al. (2005) reported increase of milk production of 14.6 and 12.5%, respectively, for cows fed glycerol at 300 and 500 ml/day in 10 weeks of lactation measurement. Furthermore, feeding glycerol increased ratio of acetate to propionate in the rumen without altering the digestibility in the total tract (Remond et al., 1993; Schroder and Sudekum, 1999).

Availability of glycerine depends on the amount of biodiesel produced. To produce glycerine 1000kg of vegetable oil is mixed with 100kg of methanol which then results in 1000kg of biodiesel (methyl ester) and 100kg of crud glycerine. Typical composition of crud glycerine is about 80 percent glycerine, 10-15 percent water, with the rest being ashes, mainly salts (Sodium or Potassium salts). Whereas glycerine may be a by-product for biodiesel industry, it is a valuable ingredient for the feed producer. The net energy value of

glycerine for ruminants is 9.5 MJ/kg. (so after deduction of ruminal fermentation losses). For pigs and poultry he came to 17.6 MJ/kg. Several researches concluded the energy content of glycerine to be equal or higher than the energy content of corn. It seems that determining the correct nutritional value of glycerine is not an easy task, as it depends on animal specie and the amount used in the diet (De Haan, 2008).

Experiments on the effect of glycerine on ruminal fermentation show various results. In general, the amount of propionic acid and butyric acid increases and acetic acid decreases. It can therefore be said that glycerine can increase energy production from ruminal fermentation. Thus part of the glycerine is available as a quick source of energy which in turn reduces the negative energy balance. This makes glycerine a good alternative for propylene glycol to reduce the risk of ketosis in cows. In calf milk replacer's glycerine can act as an alternative for lactose. Research at the University of Illinois in the US measured the effect of 15% glycerine in the diet. Total energy content of the trial group and the control group were the same. The researches found no difference in growth and health, and concluded at least 37 percent of total lactose content in calf milk replacers can be replaced. Glycerine cannot be spray dried with the other ingredients such as whey powder, and therefore has to be added when preparing the milk. In conclusion many studies found that glycerine is a welcome feed ingredient for cattle, pigs and poultry, its use depends mainly – as any other feedstuff – on its price. The price is determined by political and economical developments, which are never a stable factor (De Haan, 2008). Although glycerin is generally recognized as safe for use in animal feed (FDA, 2006), but there is little information available on glycerol feeding rates and production responses in sheep.

3. AIM OF THE STUDY:

- Using of glucoplastics substance (glycerol) as an energy supplement in nutrition.
- Study the glycerol effect on reproduction performance in ewes.
- Investigate the changes of reproduction hormones levels in breeding season.
- Studying the effect of glycerol as an energy supplement on enzymes variations in blood.
- Investigate metabolic changes in blood (proteins, carbohydrate, fats and minerals).
- Study the variation of haematology.
- Comparing different levels of glycerol used in ewes diet when we using glycerol as an energy replacement in nutrition too.

4. MATERIALS AND METHODS:

For verification the impact of a glucoplastic substance (Glycerol) on reproduction performance in ewes, this experiment was conducted in Žirany research farm with 48 cross-bred ewes (IDF X MERINO) including two trials; (I) as a replacement energy and (II) as an energy supplement in two breeding season to investigate the influence of Glycerol (which has never been studied on sheep reproduction) in nutrition on reproduction parameters and evaluation hormonal changes, fat metabolism, proteins, minerals profile in blood and haematology characteristics of ewes in breeding season. We were used crude glycerol 80% (Pic,1…Appendix) on feed ration before one month and during breeding season (June-September).

4.1 GLYCEROL CHARACTERISTICS.

Glycerol is a chemical compound also commonly called glycerin or glycerine. It is a colorless, odorless, viscous liquid that is widely used in pharmaceutical formulations. Glycerol has three hydrophilic hydroxyl groups that are responsible for its solubility in water and its hygroscopic nature. The glycerol substructure is a central component of many lipids. It is sweet-tasting and of low toxicity. Glycerol is a 10% by-product of biodiesel production. As a food additive, glycerol is also known as E number E422. Glycerol can also be used as a bodybuilding supplement, and increases energy production from ruminal fermentation (De Haan, 2008), also the cost of glycerol is cheaper. A chemical analysis (table 1) for glycerol was conducted from (Commodity Trading.s.r.o).

Chemical analyses for Glycerol.

Table 1

	Unit	Value	Limit
Mass weight	%	80.06	Min. 80
NaCl	%	7.15	7.0-9.0
Water	%	8.00	4.0-12.0
Methanol	%	0.001	Max. 0.5
Net energy	MJ/Kg	13	

4.2 STUDY DESIGN.

These trials are set up to be test as depended on the background what we obtained from previous experiments which we conducted in the Žirany farm.

4.2.1 TRIAL (I).

Forty eight ewes (4-7 years old) with a body weight ranged from 58-73 kg were assigned randomly into three groups and one control (each group 12 ewes) (Pic,5....Appendix).One ram was used for natural mating in estrus cycle for groups ewes. Three different levels of energy that content a feed ration by (NRC,2007) in requirement for ewes were fed one month before the breeding season to each of the three groups respectively, 1st group (100% forage energy), 2nd group (70% forage energy) and 3rd group (80% forage energy + 20% glycerol energy). Control group (4th) was grazed only on pasture having ad libitum food during experiment period (breeding season). Feed rations were analyzed for net energy, crud protein, crud fiber and dry mater in Department of Animal Nutrition in Faculty of Agrobiology and Food Resources at the Slovak University of Agriculture in Nitra (SUA) (Table 2, 3, 4, 5).

Chemical analyses for nutrient composition, trial (I)

Table 2

	Net energy-MJ	**Crud protein g\kg**	**Crud fiber %**	**Dry matter %**
Corn silage	6.15	9.16	21.53	33.29
Meadow hay	4.46	9.5	33.0	75.01
Alfalfa hay	4.67	20.58	25.7	84.38
Glycerol	13	------	--------	--------

Feed ration of 1st group (100% energy)

Table 3

	Kg of orig. mass	**100% of dry matter in kg**	**NEL-MJ**	**PDI-g**	**NL- g/kg**	**Crud fiber %**
Rate for ewe 70 kg weigh		1.4	5.5	64	102	350
Corn silage	0.65 (7.8k)	0.216	1.32	11.93	19.69	46.29
Meadow hay	0.8 (9.6k)	0.6	2.68	35.4	57	198
Alfalfa hay	0.5 (6.0k)	0.422	2.01	36.42	88.49	110.51
In feed ration		1.24	6.01	83.75	165.19	354.8
Requirement		1.4	5.5	64	102	350
Difference		-0.16	0.51	19.75	63.19	4.8

> Minerals add as libitum

Feed ration of 2nd group (70% energy)

Table 4

	Kg of orig. mass	100% of dry matter in kg	NEL-MJ	PDI-g	NL- g/kg	Crud fiber %
Rate for ewe 70 kg weigh		1.4	5.5	64	102	350
Corn silage	0.45 (5.4k)	0.15	0.92	8.31	13.73	32.27
Meadow hay	0.6 (7.2k)	0.45	2.01	26.55	42.75	148.5
Alfalfa hay	0.4 (4.8k)	0.34	1.57	28.58	69.46	86.72
In feed ration		0.95	4.5	63.14	125.93	267.49
Requirement		1.4	5.5	64	102	350
Difference		-0.45	-1.00	-0.86	23.93	-82.51

>Minerals add as libitum

Feed ration of 3rd group (80% natural+20% glycerol)

Table 5

	Kg of orig. mass	100% of dry matter in kg	NEL-MJ	PDI-g	NL- g/kg	Crud fiber %
Rate for ewe 70 kg weigh		1.4	5.5	64	102	350
Corn silage	0.20 (2.4k)	0.067	0.41	3.69	6.09	14.34
Meadow hay	0.7 (8.4k)	0.525	2.34	30.97	49.88	173.25
Alfalfa hay	0.5 (6.0k)	0.422	2.01	36.42	88.49	108.42
Glycerol (80%)	0.10 (1.2k)		1.3			
In feed ration		1.14	6.06	71.08	144.46	296.01
Requirement		1.4	5.5	64	102	350
Difference		-0.26	0.56	7.08	42.46	-53.99

> Minerals add as libitum

4.2.2 TRIAL (II).

Also forty eight ewes (5-7 years old) with a body weight ranged from 62-75 kg were assigned randomly into four equal groups (each group 12 ewes). The animals of trial were fed on corn silage (0.5 kg\day\ewe), meadow hay (0.8 kg\day\ewe) and alfalfa hay (0.4 kg\day\ewe) (Pic,2….Appendix). The treatments were: groups 1st , 2nd and 3rd with 150 ml (30%), 100ml (20%) and 50 ml (10%) dry matter energy of glycerol\ewe\day respectively that provided diluents with water (5 litter\ewe\day), and 4th group (control) without glycerol. The level of energy that contents a feed ration by (NRC, 2007) in requirement for ewes were fed one month before the breeding season to each of animal groups. Feed rations were analyzed for net energy, crud protein, crud fiber and dry mater in Department of

Animal Nutrition in Faculty of Agrobiology and Food Resources at the Slovak University of Agriculture in Nitra (SUA) (Table 6, 7, 8).

Chemical analyses for nutrient composition, trial (II)

Table 6

	Net energy-MJ	**Crud protein g\kg**	**Crud fiber %**	**Dry matter %**
Corn silage	6.26	6.44	15.83	42.15
Meadow hay	4.49	8.29	29.73	84.34
Alfalfa hay	4.71	15.49	38.67	91.84
Glycerol	13	------	--------	--------

Feed ration for trial (II) groups

Table7

	1st group	**2nd group**	**3rd group**	**4th group (control)**
Corn silage	0.5 kg (6kg)	0.5 kg (6kg)	0.5 kg (6kg)	0.5 kg (6kg)
Meadow hay	0.8 kg (9.6 kg)	0.8 kg (9.6 kg)	0.8 kg (9.6 kg)	0.8 kg (9.6 kg)
Alfalfa hay	0.4 kg (4.8 kg)	0.4 kg (4.8 kg)	0.4 kg (4.8 kg)	0.4 kg (4.8 kg)
Glycerol	150 ml (1.8 L)	100 ml (1.2 L)	50 ml (0.6 L)	0 ml

> Minerals add as libitum

Trial (II) nutrition components

Table 8

	Dry matter-kg	**Net energy-Mj**	**Total protein-g**	**Crude fiber-kg**
Require	1.4	5.5	102	0.41
Meadow hay	0.67	3.01	55.54	0.199
Alfalfa hay	0.37	1.74	57.31	0.143
Corn silage	0.15	0.94	9.66	0.024
Total	1.19	5.69	122.51	0.366
Difference	-0.21	0.19	20.51	-0.044

4.3 HOUSING AND MANAGEMENT.

The animals housing in the same administration, environmental condition (temperature and humidity) and area (40 m^2/ each group) (Pic,6…Appendix). Animals feeding on corn silage, alfalfa hay and meadow hay, according to feed ration (requirement) for each groups, and minerals added libitum. Animals were maintained at ambient temperature and natural day length in covered. Clean water was provided all the times. Feed rations were offered to all animals once at 08:00h that was analyzed for net energy, crud protein, crud fiber and dry mater in Department of Animal Nutrition in Faculty of Agrobiology and Food Resources at the Slovak University of Agriculture in Nitra (SUA).

4.4 THE STUDIED CHARACTERISTICS.

4.4.1 REPRODUCTION PARAMETERS.

4.4.1.1 DAY OF ESTRUS.

Ewes tested by ram 2x per day in 12 hours intervals-at 6:00 am and 6:00 pm. 1st positive reaction of ewe on ram contact qualified as beginning of estrus.

4.4.1.2 ESTRUS LENGTH.

A length of estrus calculated by the period from first positive ewes reaction – contact reflex, but without mating to last mating. Into 12 hours period we were calculated with 6 hours time allowance for start of estrus and end of estrus too.

Proceeding:

a. When we found at 6:00 am of the restraining ewe to mating, but she is mating at 6:00 pm, we calculated ending of estrus at 12:00 pm.

b. When we found at 6:00 pm of the restraining ewe to mating, but she is mating at 6:00 am, we calculated ending of estrus at 12:00 am.

c. When we found at 6:00 am of ewe with standing and she is mating, we calculated the start of estrus at12:00 pm.

d. When we found at 6:00 pm of ewe with standing and she is mating, we calculated the start of estrus at 12:00 am.

4.4.1.3 GRAVIDITY.

The ewes gravidity was tested by ultrasonography (Digital ultrasonic imaging system, Model, DP 3300 Vet, GmbH, Germany) on 30th -40th day of gravidity.

4.4.1.4 PREGNANCY LENGTH.

We calculated from mating day till lambing day.

4.4.1.5 NUMBER OF LAMBS (MALE & FEMALE).

The numbers and sex (male or female) of lambs was recorded, also the quality of parturition (singles, twins or triplet) was recorded after lambing.

4.4.1.6 WEIGHT OF NEONATAL LAMBS.

The weights of neonatal lambs were measured by an accurate scale in kilograms (kg) at the next day of the lambing.

4.4.1.7 EWES FERTILITY PERCENTAGE.

The percentage of fertility was calculated by the equation below according by Ptaszynska, (2006).

$$\text{Fertility (\%)} = \frac{\text{Number of ewes lambing}}{\text{Number of ewes exposed to the ram}} \times 100$$

4.4.1.8 EWES PROLIFICACY PERCENTAGE.

The percentage of prolificacy was calculated by the equation below according by Ptaszynska, (2006).

$$\text{Prolificacy (\%)} = \frac{\text{Number of lambs born (dead \& alive)}}{\text{Number of ewes lambing}} \times 100$$

4.4.1.9 EWES FECUNDITY PERCENTAGE.

The percentage of fecundity was calculated by the equation below according by Ptaszynska, (2006).

$$\text{Fecundity (\%)} = \frac{\text{Number of lambs born (dead \& alive)}}{\text{Number of ewes exposed to the ram}} \times 100$$

4.4.2 BLOOD PARAMETERS.

The samples of blood were collected (10 ml) from jugular vein puncture of animals (Pic,3….Appendix), at the start of experiment, beginning of estrus cycle (for hormonal and haematology evaluations) and at the end of experiment (trial I), and only at the start of experiment and at the end of experiment for trial II. Blood samples were centrifuged at 3000 x rpm for 20 min, after then the serums were separated and stored frozen at -18 ^{o}C for subsequent analysis.

4.4.2.1 CONCENTRATION OF HORMONES.

For hormones inspections was taken readers of samples by Elisa apparatus (METERTECH ∑ 960) (Pic,7….Appendix) after using a special kit for each hormone below:

4.4.2.1.1 FOLLICLE STIMULATING HORMONE (FSH).

Follicle stimulation hormone was determined according to the (FSH) kit method (FSH ELISA, Cat. No.EIA-1288.DRG Instruments GmbH, Germany) was used. Measurements were done at wavelength 450 nm.

4.4.2.1.2 LUTEINIZING HORMONE (LH).

Samples were analyzed for leutinizing hormone determined according to the (LH) kit method (LH ELISA Serum, Cat.No. EIA-1289.DRG Instruments GmbH, Germany) was used. Measurements were done at wavelength 450 nm.

4.4.2.1.3 ESTRADIOL.

Estradiol hormone was determined according to the estradiol kit method (Estradiol ELISA, Cat. No. EIA-2693. DRG Instruments GmbH, Germany) was used. Measurements were done at wavelength 450 nm.

4.4.2.1.4 PROGESTERONE.

Samples were analyzed for progesterone hormone determined according to the progesterone kit method (Progesterone ELISA, Cat. No. EIA-1561. DRG Instruments GmbH, Germany) was used. Measurements were done at wavelength 450 nm.

4.4.2.1.5 PROLACTIN.

Prolactin hormone was determined according to the prolactin kit method (Prolactin ELISA, Cat. No. EIA-1291. DRG Instruments GmbH, Germany) was used. Measurements were done at wavelength 450 nm.

4.4.2.2 BIOCHEMICAL ANALYSIS.

For biochemical inspection were taken readers for samples by Spectrophotometer apparatus (SCHOTT Instrument, Uviline 9400) (Pic,8…Appendix) after using special kits for each biochemical parameters that show below, (except sodium and potassium).

4.4.2.2.1 ALKALINE PHOSPHATASE (ALP).

Alkaline phosphatase was determined according to the (ALP) kit method (Bio LA TEST®, PLIVA-Lachema Diagnostika s.r.o., Karasek 1,62133 Brno, CZ. Cat. No. 10003188) was used. Measurements were done at wavelength 405 nm.

4.4.2.2.2 ALANINE TRANSAMINASE (ALT).

Samples were analyzed for alanine transaminase determined according to the (ALT) kit method (Bio LA TEST®, PLIVA-Lachema Diagnostika s.r.o., Karasek 1,62133 Brno, CZ. Cat. No. 10003298) was used. Measurements were done at wavelength 340 nm.

4.4.2.2.3 ASPARTATE TRANSAMINASE (AST).

Aspartate transaminase was determined according to the (AST) kit method (Bio LA TEST®, PLIVA-Lachema Diagnostika s.r.o., Karasek 1, 62133 Brno, CZ. Cat. No. 10003300) was used. Measurements were done at wavelength 340 nm.

4.4.2.2.4 TOTAL PROTEIN.

Samples were analyzed for total protein determined according to the total protein kit method (Bio LA TEST®, PLIVA-Lachema Diagnostika s.r.o., Karasek 1,62133 Brno, CZ. Cat. No. 10010217) was used. Measurements were done at wavelength 540nm.

4.4.2.2.5 UREA.

Urea was determined according to the urea kit method (Bio LA TEST®, PLIVA-Lachema Diagnostika s.r.o., Karasek 1, 62133 Brno, CZ. Cat. No. 10003079) was used. Measurements were done at wavelength 540nm.

4.4.2.2.6 GLUCOSE.

Samples were analyzed for glucose determined according to the glucose kit method (Bio LA TEST®, PLIVA-Lachema Diagnostika s.r.o., Karasek 1, 62133 Brno, CZ. Cat. No. 10003261) was used. Measurements were done at wavelength 500nm.

4.4.2.2.7 TOTAL CHOLESTEROL.

Samples were analyzed for cholesterol determined according to the cholesterol kit method (Bio LA TEST®, PLIVA-Lachema Diagnostika s.r.o., Karasek 1, 62133 Brno, CZ. Cat. No. 10003263) was used. Measurements were done at wavelength 500nm.

4.4.2.2.8 TRIGLYCERIDE.

Triglyceride was determined according to the triglyceride kit method (Bio LA TEST®, PLIVA-Lachema Diagnostika s.r.o., Karasek 1, 62133 Brno, CZ. Cat. No. 10003266) was used. Measurements were done at wavelength 500nm.

4.4.2.2.9 SODIUM (Na).

The level of sodium was measured by Operator's Manual method using MEDICA Easy Lyte apparatus according established procedure (NL-2513 BH The Hague, The Netherlands).

4.4.2.2.10 CHLORINE (Cl).

Samples were analyzed for chloride determined according to the (Cl) kit method (Bio LA TEST®, PLIVA-Lachema Diagnostika s.r.o., Karasek 1,62133 Brno, CZ. Cat. No. 10003276) was used. Measurements were done at wavelength 480nm.

4.4.2.2.11 POTASSIUM (K).

Potassium concentrations were measured by Operator's Manual method using MEDICA Easy Lyte apparatus according established procedure (NL-2513 BH The Hague, The Netherlands).

4.4.2.2.12 CALCIUM (Ca).

Samples were analyzed for calcium determined according to the (Ca) kit method (Bio LA TEST®, PLIVA-Lachema Diagnostika s.r.o., Karasek 1,62133 Brno, CZ. Cat. No. 10003281) was used. Measurements were done at wavelength 600 nm.

4.4.2.2.13 PHOSPHOR (P).

Phosphore was determined according to the (P) kit method (Bio LA TEST®, PLIVA-Lachema Diagnostika s.r.o., Karasek 1, 62133 Brno, CZ. Cat. No. 10003285) was used. Measurements were done at wavelength 340 nm.

4.4.2.2.14 MAGNESIUM (Mg).

Samples were analyzed for magnesium determined according to the (Mg) kit method (Bio LA TEST®, PLIVA-Lachema Diagnostika s.r.o., Karasek 1,62133 Brno, CZ. Cat. No. 10003279) was used. Measurements were done at wavelength 520nm.

4.4.2.3 HAEMATOLOGY PARAMETERS.

The samples of blood were collected (5 ml) from jugular vein puncture of animals into disposable heparins test tubes, at the start of experiment, beginning of estrus cycle and at the end of experiment (trial I), and only at the start of experiment and at the end of experiment for trial II. For hematological inspections, we were analyzed by Haematology Analyzer apparatus (Abacus Junior vet Diatron GmbH, Wien- Austria) (Pic,9….Appendix), for each parameter below:

a. WHITE BLLOD CELLS (WBC).

b. LYMPHOCYTE (LY).

c. GRANULOCYTE (GR).

d. RED BLOOD CELLS (RBC).

e. HEMOGLOBIN (HGB).

f. HEMATOCRITE (HCT).

4.5 STATISTICAL ANALYSES.

For the statistical design and data analyses, complete random design an experiment treatments were determined. Data in all experiments were subjected to (ANOVA) one way procedures appropriate for a completely randomized design (CRD) and the significance of differences between the means estimated using Duncan test (Duncan's Multiple Range Test). Probability level of $P<0.05$ was considered for significance in all comparisons. All statistical analyses were performed using the software SPSS 11.5 for Windows® (SPSS Inc., Chicago, IL).

5. RESULTS:

5.1 TRIAL (I).

5.1.1 GLYCEROL EFFECT AS REPLACEMENT ENERGY ON REPRODUTION CHARACTARESTICS.

5.1.1.1 ESTRUS LENGTH.

The effect of glycerol using as replacement energy on estrus length was significantly (P<0.05), there were significant differences between groups that recorded glycerol group lower value (31.916±5.977) than control and 1st groups respectively (35.333±3.114) (35.416±3.554) (Table 9).

5.1.1.2 GRAVIDITY.

There were insignificant (P<0.05) differences between groups for gravidity which founded in 1st and 3rd groups were higher (1.000±0.000) than 2nd and control groups (0.916±0.289) (Table 9).

5.1.1.3 PREGNANCY LENGTH.

Pregnancy length affected by glycerol insignificantly (P<0.05) that registered a highest value in 3rd group (148.416±1.443) than respective groups; 1st (148.333±2.015), 2nd group (136.500±43.013) and control group (136.583±43.073) (Table 9).

5.1.1.4 NUMBER OF LAMBS.

Also number of lambs affected by glycerol insignificantly (P<0.05), in 3rd group was found a higher value (1.666±0.492) than other respective groups; (1.416±0.515) in 1st and (1.333±0.651) in 2nd and control groups (Table 9).

Glycerol effect as energy replacement in diet on some reproduction parameters

Table 9

Group	Estrus length/h	Gravidity/ewe	Pregnancy length / day	No. of lambs/ ewe
Group 1	35.416±3.554 b	1.000±0.000	148.333±2.015	1.416±0.515
Group 2	30.500± 7.180 a	0.916±0.289	136.500±43.013	1.333±0.651
Group 3	31.916±5.977 ab	1.000±0.000	148.416±1.443	1.666±0.492
Group4,(Control)	35.333±3.114 b	0.916±0.289	136.583±43.073	1.333±0.651

[a b] Means values with different superscripts within a column differ significantly (P<0.05)

5.1.1.5 SINGLE LAMBING.

Obtained data on single lambing are presented in table (10). Insignificant (P<0.05) differences between groups were observed, which decreased in glycerol group (0.333±0.492) comparison with other groups; (0.583±0.515) in 1st group and (0.500±0.522) in 2nd and control groups.

5.1.1.6 TWINS LAMBING.

Twins lambing results indicate insignificantly (P<0.05) differences between groups, while glycerol group was recorded highest value (0.666±0.492) than other groups equally (0.416±0.515) (Table 10).

5.1.1.7 MALE LAMBS.

Insignificantly (P<0.05) differences between groups were founded for male lambs, whereas a highest value was recorded in control group (0.916±0.669), but glycerol group was higher (0.750±0.622) than 1st group (0.416±0.669) (Table10).

5.1.1.8 FEMALE LAMBS.

There were significantly (P<0.05) differences between groups for female lambs, that showed the results in 1st and glycerol groups were higher (1.000±0.603) (0.916±0.515) than 2nd and control groups respectively (0.583±0.515) (0.416±0.515) (Table 10).

Glycerol effect as energy replacement in diet on lambing and sex results

Table 10

Group	Single lambing No./ewe	Twins lambing No./ewe	Male lambs No./ewe	Female lambs No./ewe	
Group 1	0.583±0.515	0.416±0.515	0.416±0.669	1.000±0.603	b
Group 2	0.500±0.522	0.416±0.515	0.750±0.622	0.583±0.515	ab
Group 3	0.333±0.492	0.666±0.492	0.750±0.622	0.916±0.515	b
Group4,(Control)	0.500±0.522	0.416±0.515	0.916±0.669	0.416±0.515	a

[a b] Means values with different superscripts within a column differ significantly (P<0.05)

5.1.1.9 MALE LAMBS WEIGHT.

Variance analyses of data for male lambs weight revealed no significant (P<0.05) differences between groups in table (11) that showed control group was highest (3.200±2.321) than other groups. Whereas the weight of male lambs in glycerol (3rd) group was higher (2.925±2.479) than 1st and 2nd groups respectively (1.558±2.612) (2.716±2.217).

5.1.1.10 FEMALE LAMBS WEIGHT.

In table (11), the data showed there were significant (P<0.05) differences between groups that recorded 1st and glycerol groups highest values (3.841±2.248) (3.733±2.036) respectively than 2nd and control groups (1.983±1.792) (1.575±1.956).

5.1.1.11 TOTAL LAMBS WEIGHT.

Differences between groups was significantly (P<0.05) higher in 3rd group (6.658±1.677) than other groups which observed for total lambs weight that is mean affected by glycerol (Table 11).

Glycerol effect as energy replacement in diet on lambs weight

Table 11

Group	Male lambs weight Kg/ewe	Female lambs weight (kg/ewe)	Total lambs weight Kg/ewe
Group 1	1.558±2.612	3.841±2.248 b	5.400± 1.644 ab
Group 2	2.716±2.217	1.983±1.792 a	4.700±2.181 a
Group 3	2.925±2.479	3.733±2.036 b	6.658±1.677 b
Group4,(Control)	3.200±2.321	1.575±1.956 a	4.775±2.314 a

[a b] Means values with different superscripts within a column differ significantly (P<0.05)

5.1.1.12 FERTILITY.

There were insignificant (P<0.05) differences between groups for fertility percentage, that founded in glycerol and 1st groups were higher (100.000±0.000) than 2nd and control groups (91.666±28.868) (Table 12).

5.1.1.13 PROLIFICACY.

Glycerol effected on prolificacy percentage insignificantly (P<0.05) that increased in 3rd (glycerol) group (166.666±49.237) (Table 12).

5.1.1.14 FECUNDITY.

Differences between groups for fecundity percentage were insignificantly (P<0.05), and in 3rd (glycerol) group was higher (166.666±49.237) than other groups (Table 12).

Glycerol effect as energy replacement in diet on reproduction efficiency

Table 12

Group	Fertility %	Prolificacy %	Fecundity %
Group 1	100.000±0.000	141.666±51.493	141.666±51.493
Group 2	91.666±28.868	145.454±71.055	133.333±65.134
Group 3	100.000±0.000	166.666±49.237	166.666±49.237
Group4,(Control)	91.666±28.868	145.454±71.055	133.333±65.134

5.1.2 GLYCEROL EFFECT AS REPLACEMENT ENERGY ON HORMONES.

5.1.2.1 FOLLICLE STIMULATING HORMONE (FSH).

Effects of glycerol on follicle stimulation hormone represented in figure (1). Glycerol effected FSH insignificantly (P<0.05) that decreased at the three stages (start, estrus and at the end) of experiment respectively (0.261 mlU\ml) (0.300 mlU\ml) (0.430 mlU\ml), addition to decreased in 1st group of estrus phase. Generally FSH concentration in 1st (0.257 mlU\ml) and 4th (0.333 mlU\ml) groups were decreased in the estrus phase then increased at the end of experiment respectively (0.462 mlU\ml) (0.475 mlU\ml), while in 2nd (0.398 mlU\ml) and 3rd (0.300 mlU\ml) groups were increased gradually in the estrus and at the end of experiment respectively (0.540 mlU\ml), (0.430 mlU\ml).

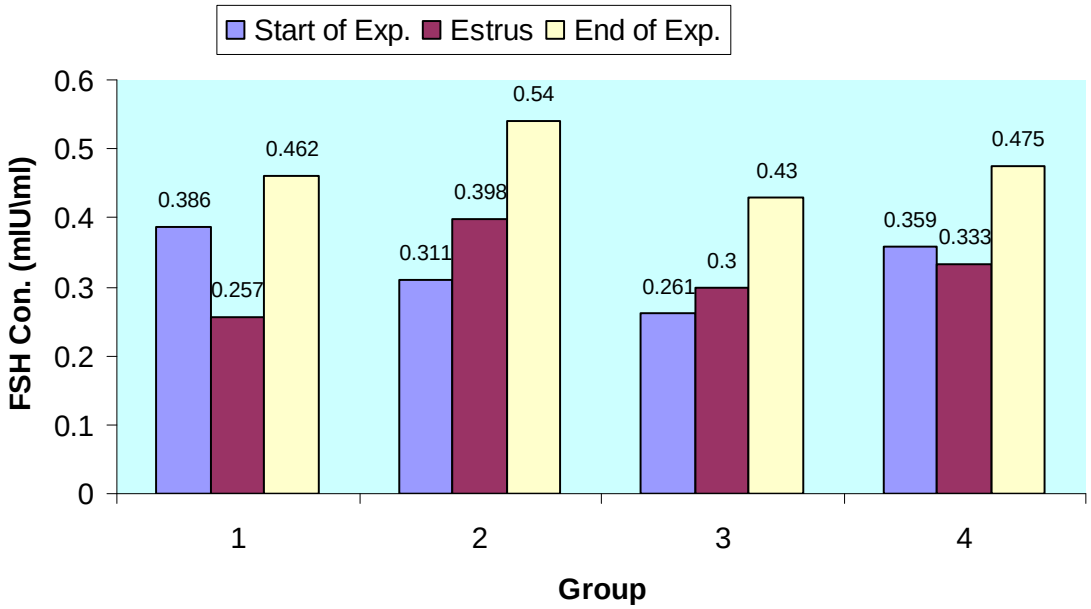
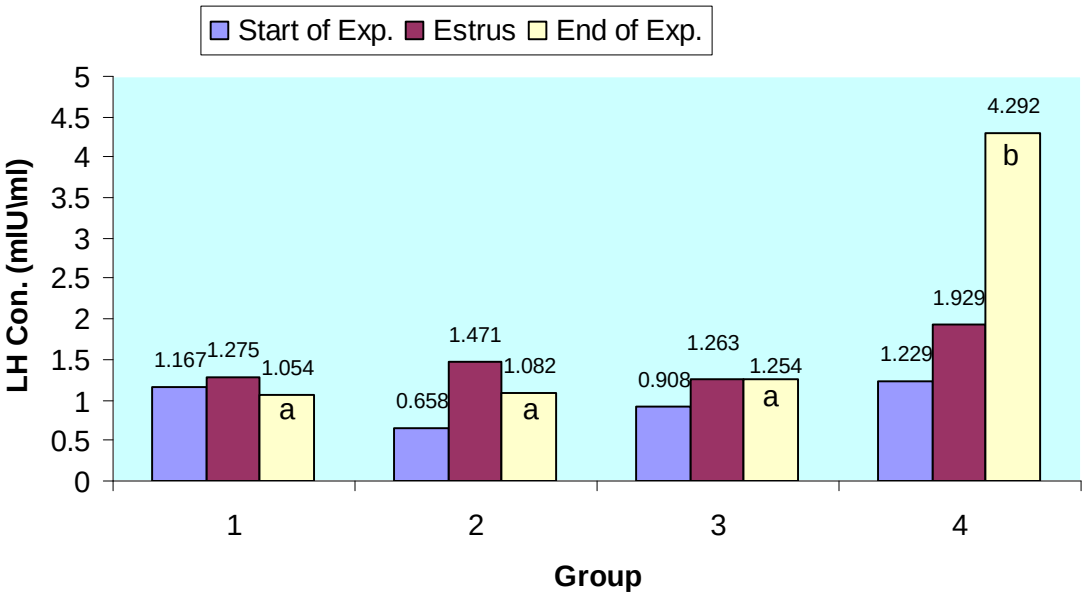


Figure 1: Glycerol as replacement energy effect on FSH

5.1.2.2 LUTEINIZING HORMONE (LH).

There were insignificant (P<0.05) differences between groups in two stages (start and estrus phase) of experiment, and significantly (P<0.05) differences between groups at the end of experiment was observed for LH concentration that showed in figure (2). The results of LH indicate insignificantly (P<0.05) effect of glycerol on LH concentration that decreased in 3rd group (1.263 mlU\ml) at the estrus phase. LH concentration in respective groups1st (1.275 mlU\ml), 2nd (1.471 mlU\ml) and 3rd (1.263 mlU\ml) were increased in estrus phase then decreased at the end of experiment respectively (1.054 mlU\ml), (1.082 mlU\ml), (1.254 mlU\ml). LH concentration for control group were higher insignificantly (P<0.05) in the start and estrus phase of experiment, and significantly (P<0.05) at the end of experiment than other groups.



[a b] Means values with different superscripts within a column differ significantly (P<0.05)

Figure 2: Glycerol as replacement energy effect on LH

5.1.2.3 ESTRADIOL HORMONE.

Estradiol hormone affected by glycerol insignificantly (P<0.05) that increased at the start of experiment (103.250 pg\ml) and in estrus phase (77.167 pg\ml), but not effected at the end of experiment. Generally, estradiol concentration in 1st (60.583 pg\ml), 2nd (66.633 pg\ml) and 3rd (77.167 pg\ml) groups were decreased in the estrus phase then increased at the end of experiment respectively (92.917 pg\ml),(92.917 pg\ml), (90.833 pg\ml) conversely than control group which decreased gradually in estrus phase then at the end of experiment (Figure 3).

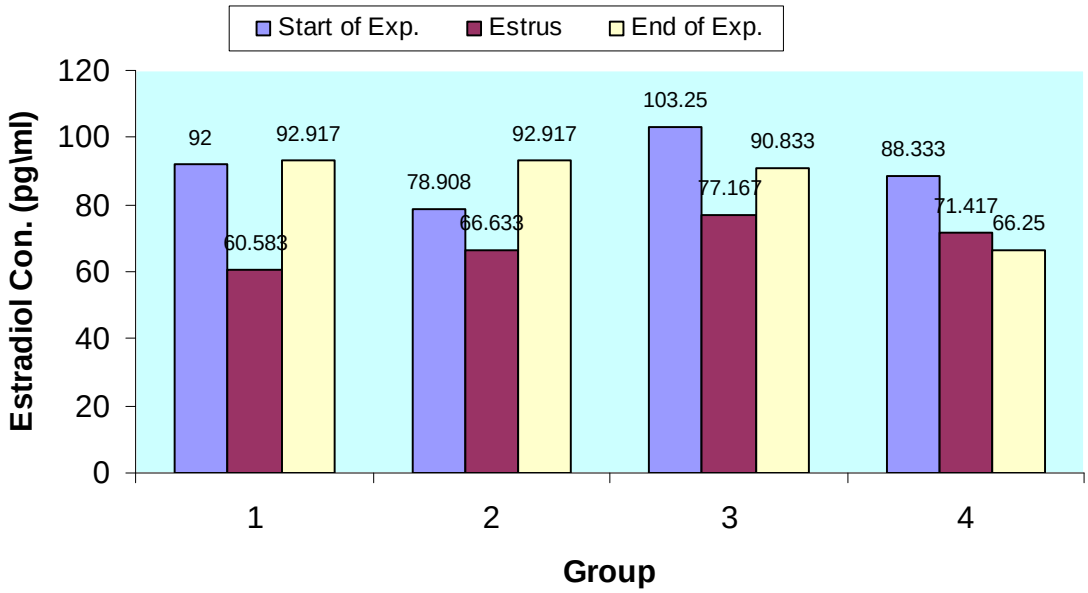
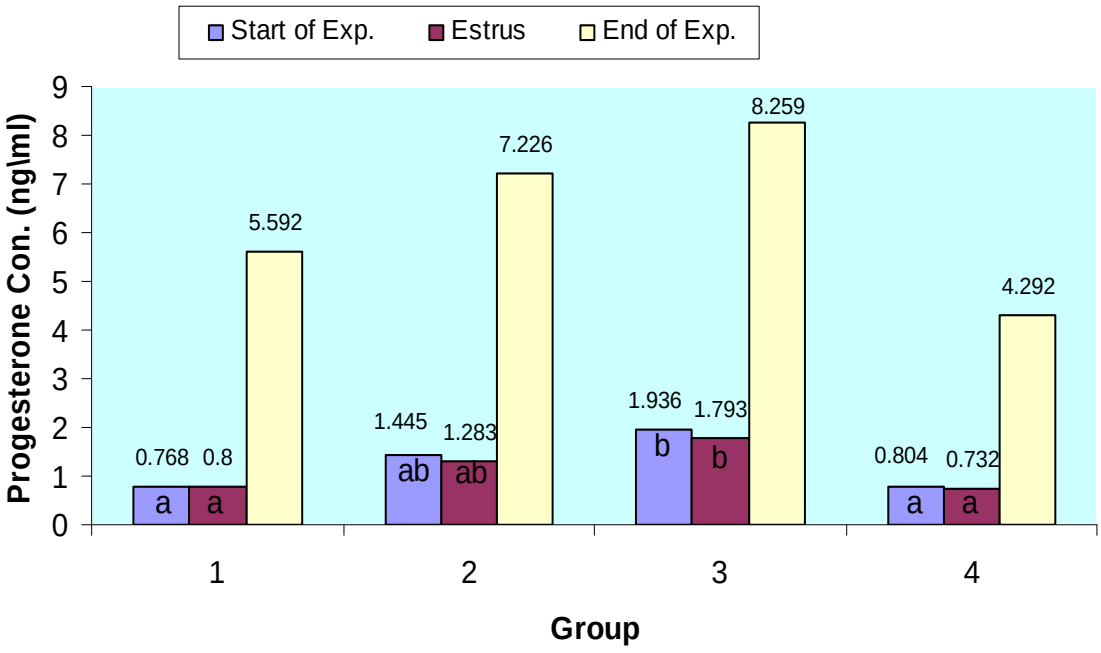


Figure 3: Glycerol as replacement energy effect on Estradiol

5.1.2.4 PROGESTERONE HORMONE.

Progesterone affected by glycerol, significantly ($P<0.05$) at the start of experiment and estrus phase, and insignificantly ($P<0.05$) at the end of experiment that recorded highest values in 3rd group for three stages respectively (1.936 ng\ml), (1.793 ng\ml), (8.259 ng\ml). We observed that glycerol group was decreased in the estrus phase then increased at the end of experiment (pregnancy period) respectively (Figure 4).

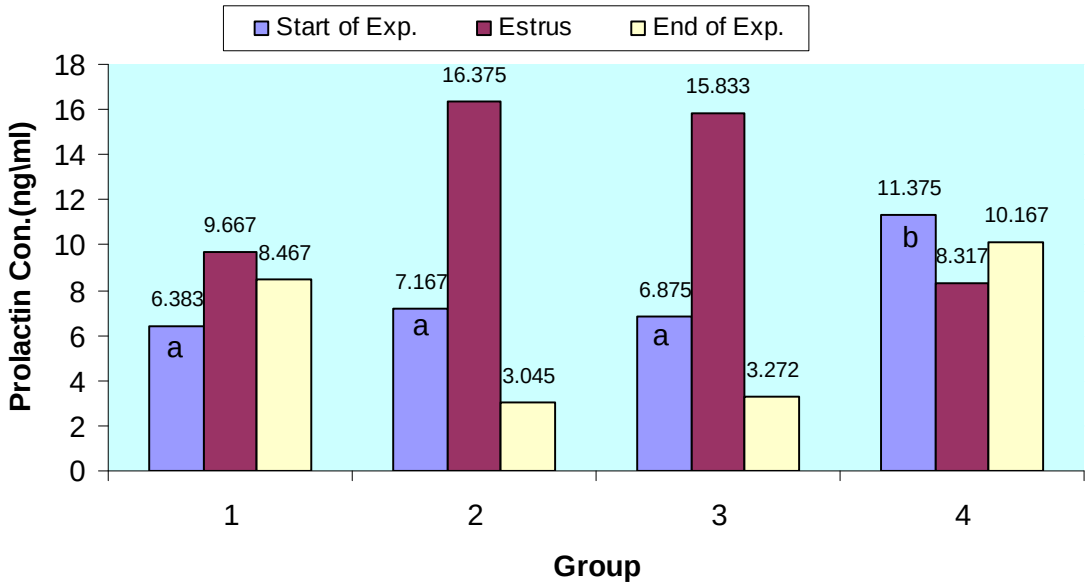


[a b] Means values with different superscripts within a column differ significantly (P<0.05)

Figure 4: Glycerol as replacement energy effect on Progesterone

5.1.2.5 PROLACTIN HORMONE.

According the results there were significant (P<0.05) differences between groups for prolactin at the start of experiment that increased in 4th group (11.375 ng\ml). There were insignificantly (P<0.05) differences between groups for prolactin in estrus and at the end of experiment. Generally prolactin concentration in respective groups 1st, 2nd and 3rd were increased in estrus phase (9.667 ng\ml), (16.375 ng\ml), (15.833 ng\ml) then decreased at the end of experiment respectively (8.467 ng\ml), (3.045 ng\ml), (3.272 ng\ml) (Figure 5).



[a b] Means values with different superscripts within a column differ significantly (P<0.05)

Figure 5: Glycerol as replacement energy effect on Prolactin

5.1.3 GLYCEROL EFFECT AS REPLACEMENT ENERGY ON BIOCHEMICAL PARAMETERS.

5.1.3.1 ALKALINE PHOSPHATASE (ALP).

According the results there were significant (P<0.05) differences between groups for ALP. In the 4th group was significantly (P<0.05) higher (2.398±1.103) than 1st (1.279±0.379) and 2nd (1.416±0.795) groups at the start of experiment, while at the end of experiment also 4th group was significantly (P<0.05) higher (2.305±0.654) than 1st group (1.383±1.131), but ALP concentration for 1st (1.383±1.131) and 2nd (1.542±1.216) groups were increased at the end of experiment (pregnancy period) comparison with the same groups at the start of experiment respectively. While ALP in 3rd (1.753±0.847) and 4th (2.305±0.654) groups at the end of experiment were lower than respective groups at the start of experiment (1.894±1.264), (2.398±1.103) (Table 13).

5.1.3.2 ALANINE TRANSAMINASE (ALT).

ALT results indicate significantly (P<0.05) differences between groups at the start and end of experiment that recorded a higher values in 4th group for two stages (start and

end) of experiment respectively (0.251±0.049) (0.274±0.043). However 3rd (glycerol) group decreased (0.188±0.033) significantly (P<0.05) at the end of experiment, while ALT in 1st (0.227±0.084) and 3rd (0.188±0.033) groups decreased at the end of experiment comparison with respective groups at the start of experiment (0.239±0.054) (0.213±0.041), but in 4th group was increased in the end of experiment in comparison with start of experiment (Table 13).

5.1.3.3 ASPARTATE TRANSAMINASE (AST).

There were significant (P<0.05) differences between groups for AST in two stages of experiment that recorded 4th group higher values in two stages (start and end) of experiment respectively (1.322±0.280) (1.543±0.340). At the end of experiment (pregnancy period), AST affected by glycerol significantly (P<0.05) that decreased in 3rd group (0.928±0.109). Generally AST concentration in 1st, 2nd and 3rd groups were higher at the start of experiment than respective groups at the end of experiment (pregnancy period), conversely than control group that increased in pregnancy period (Table 13).

Glycerol effect as replacement energy on serum enzymes

Table13

Group	ALP (µkat/l)		ALT (µkat/l)		AST (µkat/l)	
	Start of Exp.	End of Exp.	Start of Exp.	End of Exp.	Start of Exp.	End of Exp.
Group 1	1.279±0.379 a	1.383±1.131 a	0.239±0.054 b	0.227±0.048 b	1.293±0.466 b	1.058±0.138 a
Group 2	1.416±0.795 a	1.542±1.216 ab	0.193±0.042 a	0.193±0.023 a	0.988±0.148 a	0.953±0.147 a
Group 3	1.894±1.264 ab	1.753±0.847 ab	0.213±0.041 ab	0.188±0.033 a	1.118±0.268 ab	0.928±0.109 a
Group 4, Control	2.398±1.103 b	2.305±0.654 b	0.251±0.049 b	0.274±0.043 c	1.322±0.280 b	1.543±0.340 b
Normal range	0.10- 2.60		0.10- 0.40		0.40- 0.60	

[a b c] Means values with different superscripts within a column differ significantly (P<0.05)

5.1.3.4 TOTAL PROTEIN.

Total protein results indicate insignificantly (P<0.05) differences between groups that recorded in 4th group higher value than other groups at the start of experiment

(75.229±2.970), but it was be significantly (P<0.05) higher than 2nd group at the end of experiment (75.770±3.120). While the results indicate that total protein in 1st (74.378±4.515) and 4th (75.770±3.120) groups at the end of experiment was increased than same groups at the start of experiment respectively (73.884±5.522) (75.229±2.970), but in the 2nd (71.148±4.547) and 3rd (73.037±4.408) groups at the end of experiment (pregnancy period) was decreased than same groups at the start of experiment respectively (71.843±2.822) (73.712±4.885) (Table 14).

5.1.3.5 UREA.

There were insignificant (P<0.05) differences between groups for urea, in both stages of experiment, while a higher value for urea was recorded at the start of experiment in 3rd (glycerol) group(6.658±0.872), that is mean urea affected by glycerol, and 1st group at the end of experiment (6.683±0.648). From the results we observe that urea concentration in 1st (6.683±0.648) and 4th (6.050±1.257) groups were increased and in 2nd (6.167±1.204) and 3rd (6.125±0.869) groups were decreased at the end of experiment (pregnancy period) comparison with respective groups at the start of experiment (Table 14).

5.1.3.6 GLUCOSE.

Glucose affected by glycerol insignificantly (P<0.05) that increased (2.575±0.367) at the start of experiment and decreased (2.392±0.337) at the end of experiment (pregnancy period). Generally glucose in 1st, 3rd and 4th groups was decreased at the end of experiment comparison with respective groups in the start of experiment conversely than 2nd group which increased at the end of experiment (pregnancy period) (Table 14).

5.1.3.7 TOTAL CHOLESTEROL.

Total cholesterol results indicate that glycerol effected insignificantly (P<0.05) which decreased in both stages (start and at the end) of experiment respectively (1.618±0.366) (1.558±0.324). Generally, total cholesterol concentration in all groups decreased at the end of experiment (pregnancy period) comparison with respective groups in the start of experiment (Table 14).

5.1.3.8 TRIGLYCERIDE.

The results showed there were significantly ($P<0.05$) differences between groups for triglyceride in the start and at the end of experiment, which in 3rd group increased significantly ($P<0.05$) at the start of experiment (0.283±0.130), and decreased significantly ($P<0.05$) at the end of experiment (0.217±0.033). And we observed that triglyceride concentration in 2nd (0.223±0.040) and 4th (0.269±0.040) groups at the end of experiment (pregnancy period) were increased, conversely than 1st (0.230±0.088) and 3rd (0.217±0.033) groups which decreased, when compared with both groups in the start of experiment respectively (Table 14).

Glycerol effect as replacement energy on blood biochemical properties

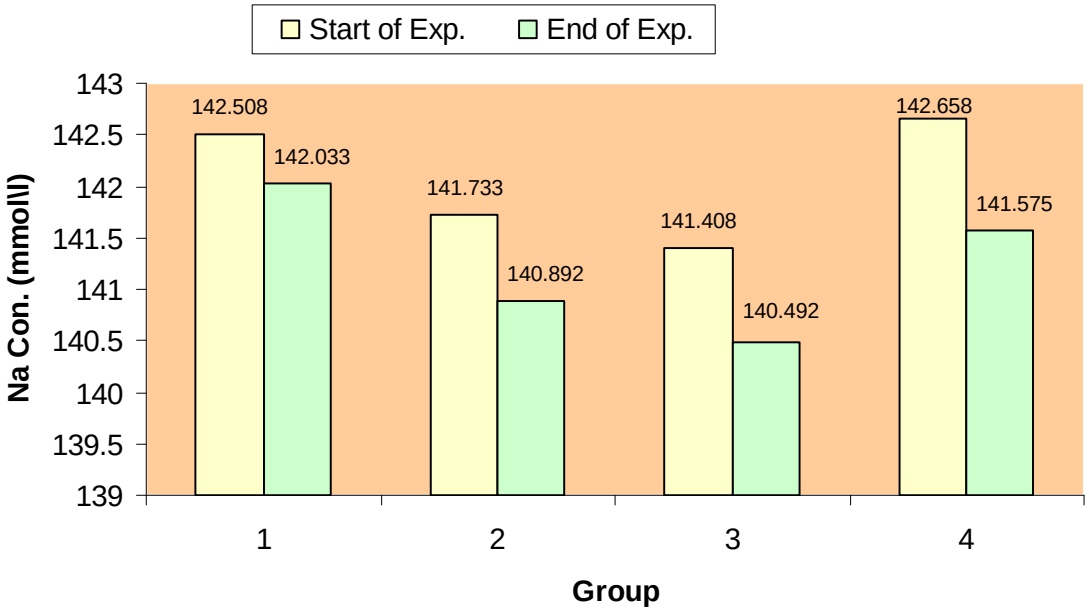
Table 14

Group	Total Protein (g/l)		Urea (mmol/l)		Glucose(mmol/l)		Cholesterol(mmol/l)		Triglyceride(mmol/l)	
	Start of Exp.	End of Exp.	Start of Exp.	End of Exp.	Start of Exp.	End of Exp.	Start of Exp.	End of Exp.	Start of Exp.	End of Exp.
Group 1	73.884±5.522	74.378±4.515 ab	6.317±1.108	6.683±0.648	2.567±0.337	2.492±0.366	1.640±0.326	1.636±0.309	0.242±0.033 ab	0.230±0.088 ab
Group 2	71.843±2.822	71.148±4.547 a	6.2250.723±	6.167±1.204	2.492±0.326	2.592±0.340	1.674±0.296	1.623±0.208	0.213±0.050 a	0.223±0.040 ab
Group 3	73.712±4.885	73.037±4.408 ab	6.658±0.872	6.125±0.869	2.575±0.367	2.392±0.337	1.618±0.366	1.558±0.324	0.283±0.130 b	0.217±0.033 a
Group4, Control	75.229±2.970	75.770±3.120 b	5.942±0.998	6.050±1.257	2.525±0.260	2.425±0.391	1.823±0.241	1.774±0.274	0.243±0.043 ab	0.269±0.040 b
Normal range	62- 86		2.8- 8.0		2.40- 4.5		1.10- 2.30		0.15- 0.50	

[a b] Means values with different superscripts within a column differ significantly ($P<0.05$)

5.1.3.9 SODIUM (Na).

The results showed sodium concentration affected by glycerol that decreased insignificantly (P<0.05) in both respective stages (start and at the end) of experiment (141.408 mmol\l) (140.492 mmol\l). And showed the Na concentrations at the end of experiment (pregnancy period) were decreased in all groups when compared with respective groups at the beginning of experiment (Figure 6).



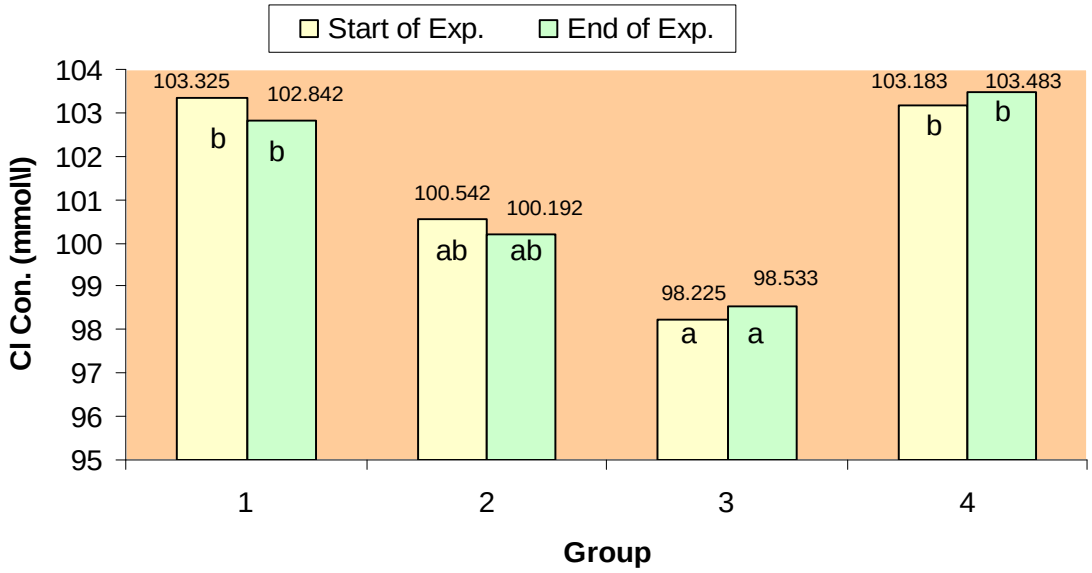
Normal range (130- 155)

Figure 6: Glycerol as replacement energy effect on serum Sodium

5.1.3.10 CHLORINE (Cl).

Cl results indicate significantly (P<0.05) differences between groups at the start and the end of experiment that recorded a significantly (P<0.05) lowest values in 3rd group for two stages of experiment respectively (98.225 mmol\l) (98.533 mmol\l). However Cl concentrations in 1st (102.842 mmol\l) and 2nd (100.192 mmol\l) groups were decreased at the end of experiment (pregnancy period) comparison with respective groups at the start of

experiment, conversely than control group which increased in pregnancy period (103.483 mmol\l) (Figure 7).

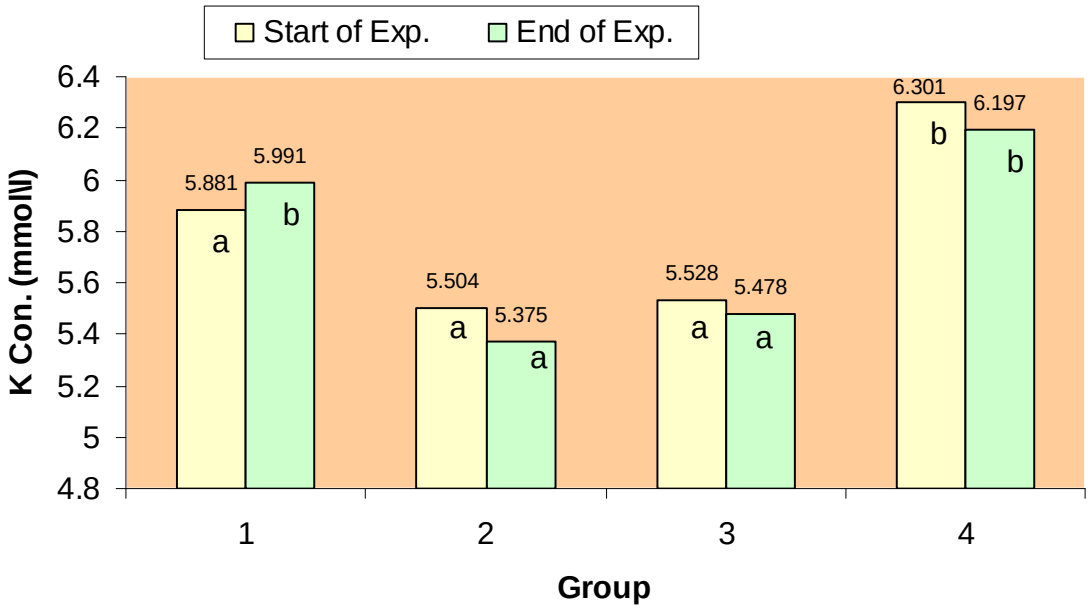


[a b] Means values with different superscripts within a column differ significantly (P<0.05)
Normal range (90- 115)

Figure 7: Glycerol as replacement energy effect on serum Chloride

5.1.3.11 POTASSIUM (K).

There were significant (P<0.05) differences between groups for K in two stages of experiment that recorded 4th group a higher value than other groups in the start and the end of experiment respectively (6.301 mmol\l) (6.197 mmol\l). While K concentrations at the end of experiment (pregnancy period) were increased in 1st (5.991 mmol\l) group and decreased in 2nd (5.375 mmol\l), 3rd (5.478 mmol\l) and 4th (6.197 mmol\l) groups when compared with respective groups at the start of experiment (Figure 8).

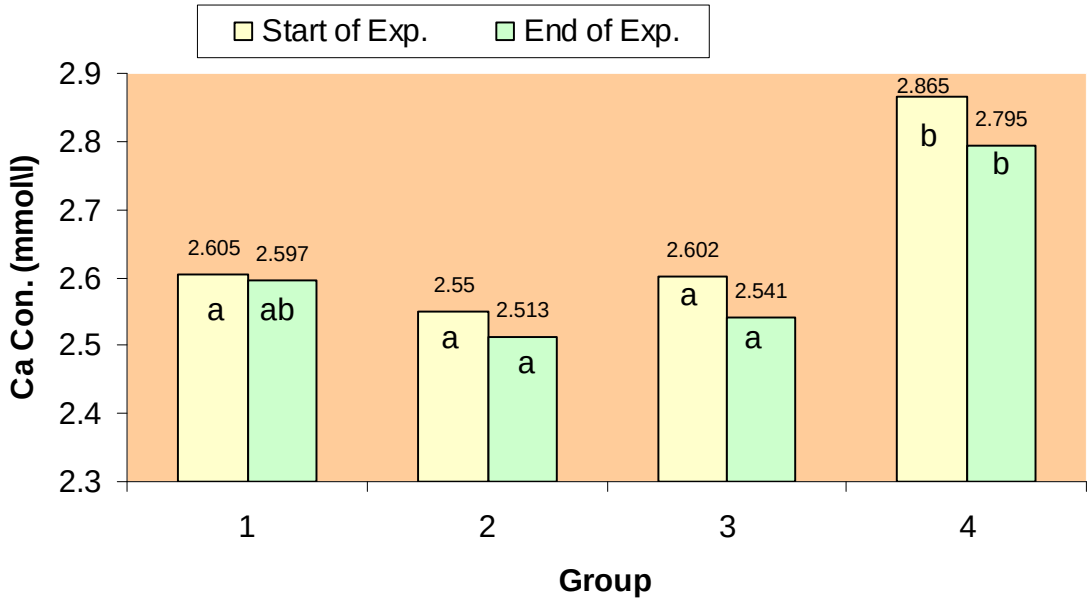


[a b] Means values with different superscripts within a column differ significantly (P<0.05)
Normal range (3.9- 6.0)

Figure 8: glycerol as replacement energy effect on serum Potassium

5.1.3.12 CALCIUM (Ca).

At the Ca results, there were significant (P<0.05) differences between groups at the start and at the end of experiment that recorded highest values in 4th group in two stages of experiment (2.865 mmol\l) (2.795 mmol\l). The Ca concentrations were decreased in all groups (beginning from 1st to control group) 2.597 mmol\l, 2.513 mmol\l, 2.541 mmol\l and 2.795 mmol\l at the end of experiment (pregnancy period) comparison with respective groups at the start of experiment (Figure 9).

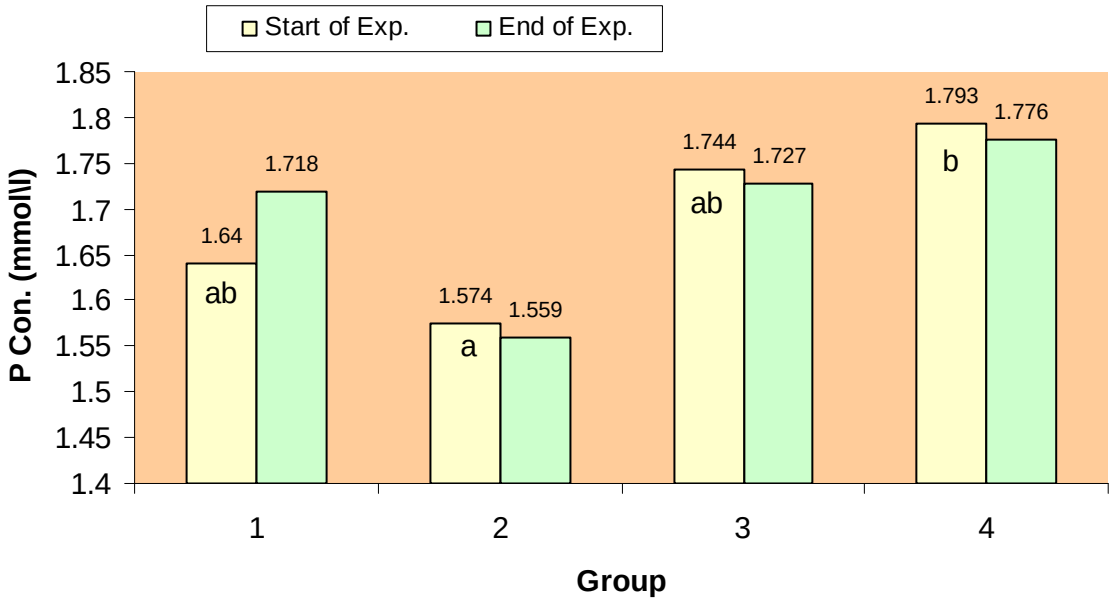


[a b] Means values with different superscripts within a column differ significantly (P<0.05)
Normal range (2.26- 3.00)

Figure 9: glycerol as replacement energy effect on serum Calcium

5.1.3.13 PHOSPHOR (P).

Significantly (P<0.05) differences between groups observed for Phosphor at the start of experiment that recorded 4th group a higher value (1.793 mmol\l). Glycerol not effected on Phosphor concentration at the end of experiment that indicate insignificant (P<0.05) differences between groups, while 4th group also registered a higher value (1.776 mmol\l). The comparison between two stages of experiment, we observed that Phosphor concentrations at the end of experiment (pregnancy period) were increased in 1st group (1.718 mmol\l) and decreased in all other groups (2nd; 1.559 mmol\l, 3rd; 1.727 mmol\l and 4th; 1.776 mmol\l) than respective groups at the start of experiment (Figure 10).

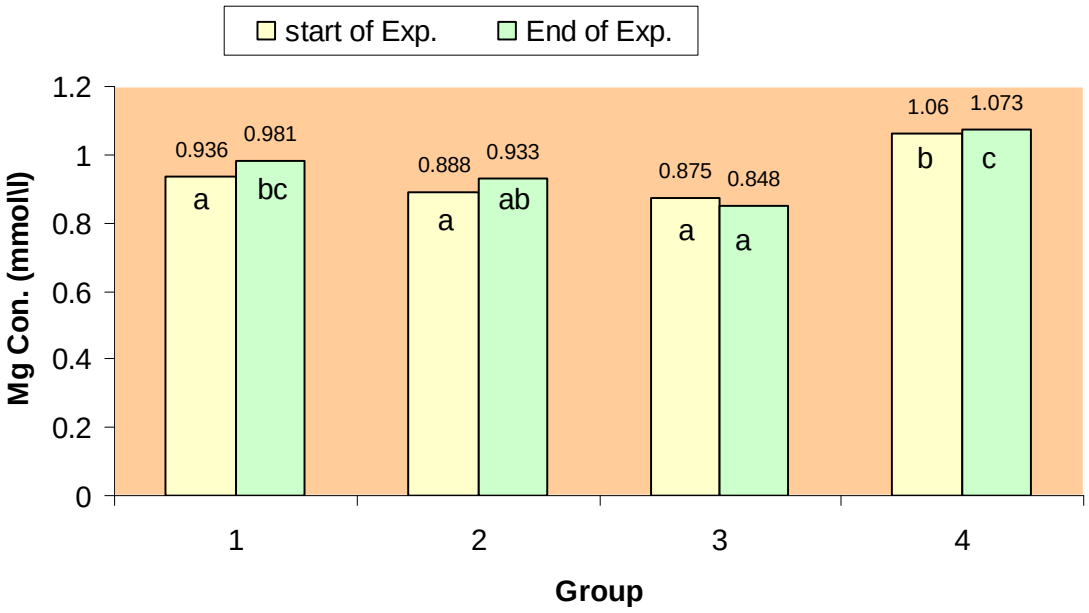


[a b] Means values with different superscripts within a column differ significantly (P<0.05)
Normal range (1.60- 2.40)

Figure 10: Glycerol as replacement energy effect on serum Phosphor

5.1.3.14 MAGNESIUM (Mg).

Mg results indicate significantly (P<0.05) differences between groups at the start and the end of experiment that recorded lowest value in 3rd (glycerol) group for two stages of experiment respectively (0.875 mmol\l) (1.073 mmol\l). However Mg concentrations at the end of experiment (pregnancy period) were decreased in 3rd group (0.848 mmol\l) and increased in 1st (0.981 mmol\l), 2nd (0.933 mmol\l) and 4th (1.073 mmol\l) groups when compared with respective groups at the start of experiment (Figure 11).



[a b c] Means values with different superscripts within a column differ significantly (P<0.05)
Normal range (0.80- 1.15)

Figure 11: Glycerol as replacement energy effect on serum Magnesium

5.1.4 GLYCEROL EFFECT AS REPLACEMENT ENERGY ON HAEMATOLOGICAL PARAMETERS.

5.1.4.1 WHITE BLOOD CELLS (WBC).

Glycerol effected on white blood cells insignificantly (P<0.05) at the start of experiment that recorded lower value in 3rd group (5.081±1.615), while in estrus phase the differences between groups were also insignificant (P<0.05) that registered 1st group lower value (6.173±1.254), whereas at the end of experiment (pregnancy period) significant (P<0.05) differences between groups were founded that recorded a lower value in 2nd group (3.346±1.235). Generally WBC groups in estrus phase were highest than respective groups in the start of experiment, then all groups were decreased at the end of experiment (pregnancy period) respectively (Table 15).

5.1.4.2 LYMPHOCYTE (LY).

Lymphocyte results indicate that is affected by glycerol at the start of experiment insignificantly ($P<0.05$) which increased in 3rd group (53.117±8.187), then insignificant ($P<0.05$) differences between groups were observed in estrus phase which increased in 2nd group (48.042±10.670), but glycerol effected LY at the end of experiment (pregnancy period) which was decreased significantly ($P<0.05$) in 3rd group (62.542±8.245). Results showed LY respective groups were decreased in estrus phase when compared with starting of experiment groups, then increased at the end of experiment (pregnancy period) respectively (Table 15).

5.1.4.3 GRANULOCYTE (GR).

Insignificant ($P<0.05$) differences between groups were founded for GR at the start of experiment which decreased in glycerol group (46.383±8.187), but glycerol was increased insignificantly ($P<0.05$) in the estrus phase (57.267±12.534), then GR affected by glycerol than increased significantly ($P<0.05$) at the end of experiment (pregnancy period). Generally all GR groups were increased in the estrus phase then decreased at the end of experiment respectively when compared with groups at the start of experiment (Table 15).

5.1.4.4 RED BLOOD CELLS (RBC).

There were insignificant ($P<0.05$) differences between groups for RBC in three stages (start, estrus and at the end) of experiment. RBC affected by glycerol which increased in the start of experiment and estrus phase respectively (12.522±0.972) (11.448±0.692), but glycerol not effected on RBC at the end of experiment (pregnancy period), while all RBC groups were decreased in estrus phase then increased at the end of experiment (pregnancy period) respectively comparison with start of experiment groups(Table 15).

5.1.4.5 HEMOGLOBIN (HGB).

Insignificant ($P<0.05$) differences between groups were founded for HGB in the three stages (start, estrus and at the end) of experiment. HGB affected by glycerol which increased at the start of experiment (126.917±10.229) and estrus phase (128.917±13.160),

whereas not effected at the end of experiment (pregnancy period). Generally three HGB groups (1^{st}, 2^{nd} and 4^{th}) were decreased in estrus phase then increased at the end of experiment respectively when compared with start of experiment groups, conversely than 3^{rd} (glycerol) group which increased in estrus phase then decreased at the end pf experiment comparison with start of experiment group (Table 15).

5.1.4.6 HEMATOCRITE (HCT).

Differences between groups for HCT were insignificantly ($P<0.05$) in all stages (start, estrus and at the end) of experiment. Glycerol effected on HCT which increased in two first stages (at the start and estrus phase) of experiment respectively (38.928±2.609) (34.120±2.383), but not effected at the end of experiment (pregnancy period). The comparison with three stages of experiment, we observe all trial groups in estrus phase were lower than start of experiment groups respectively, after then they were increased at the end of experiment (pregnancy period) (Table 15).

Glycerol as replacement energy effect on hematological parameters

Table 15

		Group 1	Group 2	Group 3	Group 4, Control	Normal range
WBC ($x10^9$\L)	Start of Exp.	5.451 ± 2.024	5.548 ± 1.861	5.081 ± 1.615	6.205 ± 2.205	5.1- 11.1
	Estrus	6.173 ± 1.254	6.987 ± 1.226	6.186 ± 1.949	7.142 ± 1.292	
	End of Exp.	4.085 ± 1.917 ab	3.346 ± 1.235 a	3.973 ± 1.235 a	5.349 ± 1.520 b	
LY (%)	Start of Exp.	52.875 ± 7.930	52.150 ± 5.734	53.117 ± 8.187	52.392 ± 9.772	32-76
	Estrus	42.000 ± 8.014	48.042 ± 10.670	42.233 ± 12.534	44.417 ± 10.033	
	End of Exp.	66.092 ± 8.411 ab	69.342 ± 6.752 b	62.542 ± 8.245 a	63.017 ± 4.649 a	
GR (%)	Start of Exp.	46.625 ± 7.930	47.350 ± 5.735	46.383 ± 8.187	47.108 ± 9.772	23- 69
	Estrus	57.500 ± 8.014	51.458 ± 10.670	57.267 ± 12.534	55.083 ± 10.033	
	End of Exp.	33.417 ± 8.402 ab	29.917 ± 6.633 a	36.958 ± 8.245 b	36.233 ± 4.682 b	
RBC ($x10^{12}$\L)	Start of Exp.	12.062 ± 1.224	11.778 ± 0.924	12.522 ± 0.972	11.848 ± 1.120	5.2- 11.2
	Estrus	10.983 ± 1.121	10.900 ± 0.762	11.448 ± 0.692	11.032 ± 1.002	
	End of Exp.	11.733 ± 0.814	11.654 ± 0.787	12.152 ± 0.728	12.282 ± 0.923	
HGB (g\L)	Start of Exp.	124.417 ± 12.965	121.500± 14.400	126.917 ± 10.229	122.667 ± 12.086	90- 140
	Estrus	122.500 ± 13.575	117.667 ± 11.578	128.917 ± 13.160	121.083 ± 12.602	
	End of Exp.	126.000 ± 11.119	127.083 ± 11.572	126.667 ± 11.711	129.500 ± 12.124	
HCT (%)	Start of Exp.	37.632 ± 3.482	36.410 ± 4.275	38.928 ± 2.609	36.762 ± 3.290	30- 40
	Estrus	32.013 ± 2.739	31.483 ± 3.535	34.120 ± 2.383	31.628 ± 3.252	
	End of Exp.	36.990 ± 3.469	37.963 ± 3.300	37.675 ± 3.443	38.692 ± 3.300	

[a b] Means values with different superscripts within a row differ significantly (P<0.05)

5.2 TRIAL (II).

5.2.1 GLYCEROL EFFECT AS SUPPLEMENT ENERGY ON REPRODUTION CHARACTARESTICS.

5.2.1.1 ESTRUS LENGTH.

Estrus length results indicate there were significant (P<0.05) differences between groups that recorded highest and lowest values in 4th (control) group (37.833±2.918) and 1st (30% glycerol) group (28.750±1.865) respectively (Table 16).

5.2.1.2 GRAVIDITY.

Insignificantly (P<0.05) differences between groups were observed for gravidity which registered in first three groups with equal values (1.000±0.000) were bigger than 4th (control) group (0.833±0.389) (Table 16).

5.2.1.3 PREGNANCY LENGTH.

Glycerol effected on pregnancy length insignificantly (P<0.05) which recorded a higher value in 3rd (glycerol 10%) group (149.250±1.288) then followed 2nd group (glycerol 20%) (148.583±1.782) and 1st group (glycerol 30%) (148.546±2.544) comparison with lower value which recorded in control group (148.333±0.985) (Table 16).

5.2.1.4 NUMBER OF LAMBS.

Number of lambs affected by glycerol 30% significantly (P<0.05) which registered a higher number in 1st group (1.636±0.674) and lower number was recorded in 4th (control) group (1.083±0.669) (Table 16).

Glycerol effect as supplement energy in diet on some reproduction parameters

Table 16

Group	Estrus length/h	Gravidity /ewe	Pregnancy length/ day	No. of lambs / ewe
Group 1	28.750±1.865 a	1.000±0.000 *	148.546±2.544 *	1.636±0.674 * b
Group 2	34.250±3.415 b	1.000±0.000	148.583±1.782	1.500±0.522 ab
Group 3	34.167±2.918 b	1.000±0.000	149.250±1.288	1.333±0.492 ab
Group4,(Control)	37.833±2.918 c	0.833±0.389	148.333±0.985	1.083±0.669 a

[a b c] Means values with different superscripts within a column differ significantly (P<0.05)

* Results from 11 ewes

5.2.1.5 SINGLE LAMBING.

Obtained data on single lambing are presented in table (17). Insignificant (P<0.05) differences between groups were observed, which decreased in 1st (glycerol 30%) (0.456±0.522) group and increased in 3rd (glycerol 10%) group (0.667±0.492).

5.2.1.6 TWINS LAMBING.

Differences between groups for twins lambing were insignificantly (P<0.05). Higher value was recorded in 2nd group (glycerol 20%) (0.500±0.522) then in 1st group (glycerol 30%) (0.455±0.522) and then in 3rd group (glycerol 10%) (0.333±0.492) while in 4th (control) group obtained on lower value (0.250±0.452) (Table 17).

5.2.1.7 TRIPLET LAMBING.

Triplet lambing results indicate insignificantly (P<0.05) differences between groups, while 1st (glycerol 30%) group was recorded highest value (0.091±0.302) than other groups equally (0.00) which not founded similar situation for other groups (Table 17).

Lambing types (No. /ewe)

Table 17

Group	Single lambing	Twins lambing	Triplet lambing
Group 1	0.456±0.522 *	0.455±0.522 *	0.091±0.302 *
Group 2	0.500±0.522	0.500±0.522	0.000±0.000
Group 3	0.667±0.492	0.333±0.492	0.000±0.000
Group4,(Control)	0.583±0.515	0.250±0.452	0.000±0.000

* Results from 11 ewes

5.2.1.8 MALE LAMBS.

There were insignificant (P<0.05) differences between groups for male lambs, that recorded a higher number of male lambs in 3rd (glycerol 10%) group (0.750±0.754) and a lower numbers was recorded in 2nd (glycerol 20%) group (0.500±0.522) (Table 18).

5.2.1.9 FEMALE LAMBS.

For female lambs, also the results indicate differences between groups were insignificantly (P<0.05). In 2nd (glycerol 20%) group was higher (1.000±0.603) than 1st (glycerol 30%) group (0.910±0.831) (Table 18).

Sex of lambing (No. /ewe)

Table 18

Group	Male lambs	Female lambs
Group 1	0.727±1.104 *	0.910±0.831 *
Group 2	0.500±0.522	1.000±0.603
Group 3	0.750±0.754	0.583±0.515
Group4,(Control)	0.667±0.651	0.417±0.669

* Results from 11 ewes

5.2.1.10 MALE LAMBS WEIGHT.

Variance analyses of data for male lambs weight revealed no significant ($P<0.05$) differences between groups in table (19) that showed 3rd (glycerol 10%) group was highest (2.733±2.758) than other groups. Whereas the weight of male lambs in 1st (glycerol 30%) group was higher (2.455±3.676) than control and 2nd (glycerol 20%) groups respectively (2.208±2.144) (1.825±1.922).

5.2.1.11 FEMALE LAMBS WEIGHT.

In table (19), the data showed there were significant ($P<0.05$) differences between groups that recorded 1st (glycerol 30%) group highest value (3.610±3.260) and lowest value was recorded in 4th (control) group (1.333±2.126).

5.2.1.12 TOTAL LAMBS WEGHT.

Differences between groups was significantly ($P<0.05$) higher in 1st (glycerol 30%) group (6.063±2.157) than other group which observed for total lambs weight that is mean affected by glycerol 30% (Table 19).

The weight (Kg) of lambs /ewe

Table19

Group	Male lambs weight	Female lambs weight	Total lambs weight
Group 1	2.455±3.676 *	3.610±3.260 * b	6.063±2.157 * b
Group 2	1.825±1.922	3.350±2.121 ab	5.175±1.903 ab
Group 3	2.733±2.758	2.183±1.978 ab	4.917±1.601 ab
Group4,(Control)	2.208±2.144	1.333±2.126 a	3.541±2.120 a

[a b] Means values with different superscripts within a column differ significantly ($P<0.05$)

* Results from 11 ewes

5.2.1.13 FERTILITY.

Insignificantly (P<0.05) differences between groups were observed for fertility which registered in first three groups with equal values (100.000±0.000) were bigger than 4th (control) group (83.333±38.925) (Table 20).

5.2.1.14 PROLIFICACY.

Glycerol 30% effected on prolificacy percentage insignificantly (P<0.05) that increased in 1st group (163.637±87.024) (Table 20).

5.2.1.15 FECUNDITY.

There were significant (P<0.05) differences between groups for fecundity which recorded equal highest percentage in two respective (1st and 2nd) groups (150.000±79.772), and a lower percentage was registered in 4th (control) group (108.333±66.856) (Table 20).

Reproduction efficiency parameters

Table 20

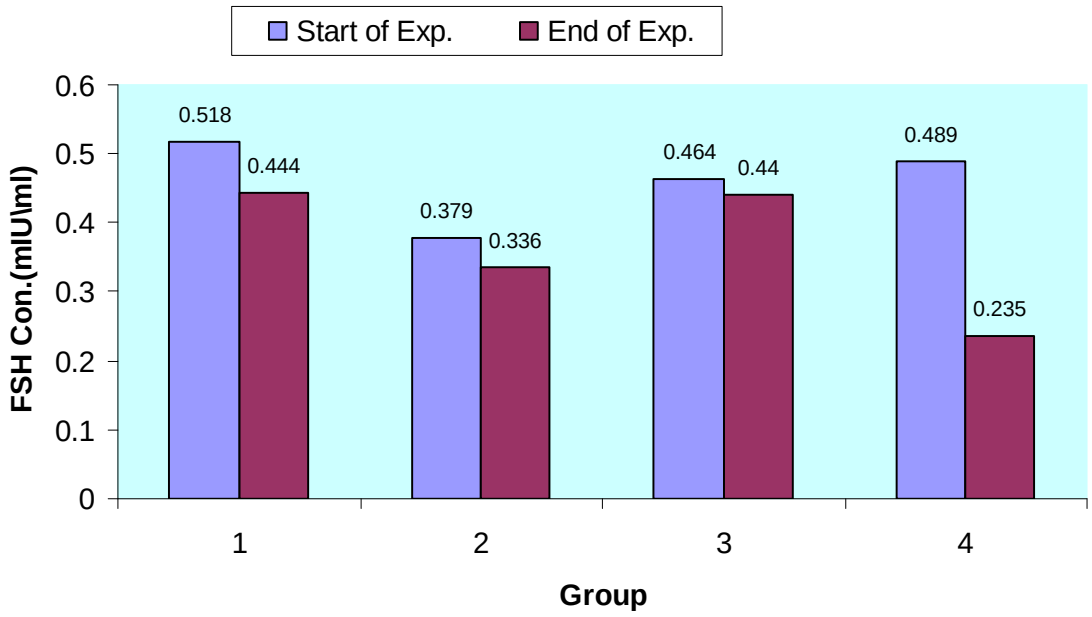
Group	Fertility %	Prolificacy %	Fecundity %
Group 1	100.000±0.000 *	163.637±87.024 *	150.000±79.772 *
Group 2	100.000±0.000	150.000±52.223	150.000±52.223
Group 3	100.000±0.000	133.333±49.236	133.333±49.236
Group4,(Control)	83.333±38.925	130.000±80.227	108.333±66.856

* Results from 11 ewes

5.2.2 GLYCEROL EFFECT AS SUPPLEMENT ENERGY ON HORMONES.

5.2.2.1 FOLLICLE STIMULATING HORMONE (FSH).

Effects of glycerol as supplement energy on follicle stimulation hormone represented in figure (12). Glycerol 30% in 1st group effected FSH insignificantly (P<0.05) that increased in two stages (at the start and end) of experiment respectively (0.518 mlU\ml) (0.444 mlU\ml) and followed 3rd group (glycerol 10%) in two respective stages (at the start and end) of experiment (0.464 mlU\ml) (0.440 mlU\ml). Generally FSH concentration in all trial groups were decreased in pregnancy period (at the end of experiment) when compared with beginning of experiment groups.

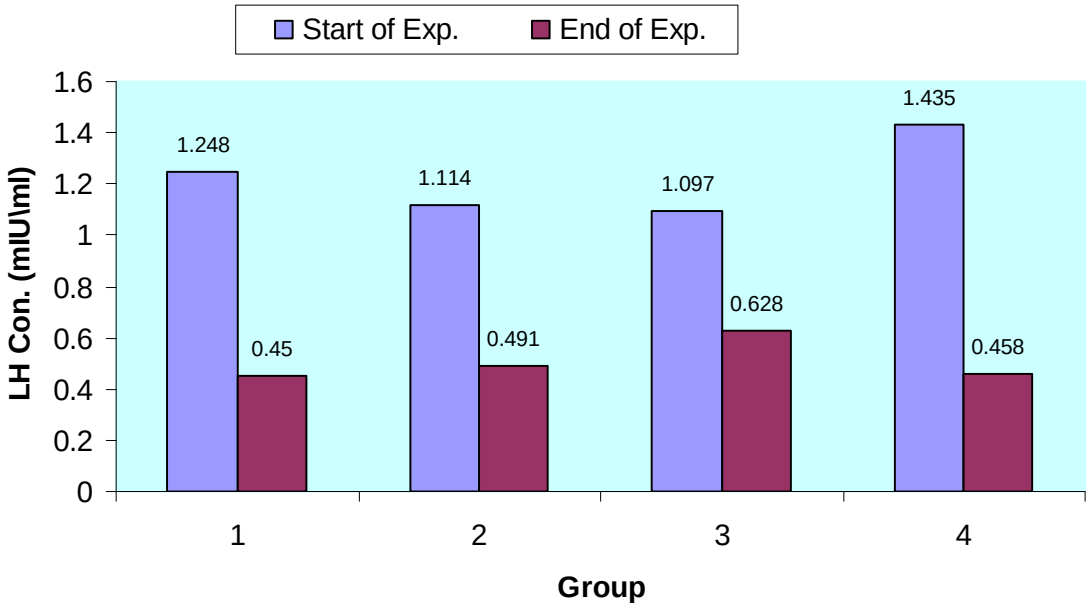


* The result for 1st group (End of Exp.) from 11 ewes

Figure 12: Glycerol as supplement energy effect on FSH

5.2.2.2 LUTEINIZING HORMONE (LH).

There were insignificant (P<0.05) differences between groups in two stages (start and at the end) of experiment which observed for LH concentration that showed in figure (13). The results of LH indicate insignificantly (P<0.05) effect of glycerol on LH concentration at the start of experiment that decreased in 3rd group (glycerol 10%) (1.097 mlU\ml) and increased in control group (1.435 mlU\ml). Conversely than at the end of experiment which observed 3rd group was recorded a higher value (0.628 mlU\ml) then 2nd group (0.491 mlU\ml), but in 1st (glycerol 30%) group LH concentration was decreased (0.450 mlU\ml). LH concentration groups at the end of experiment (pregnancy period) 1st (0.450 mlU\ml), 2nd (0.491 mlU\ml), 3rd (0.628 mlU\ml) and control (0.458 mlU\ml) were decreased comparison with respective groups at the start of experiment.

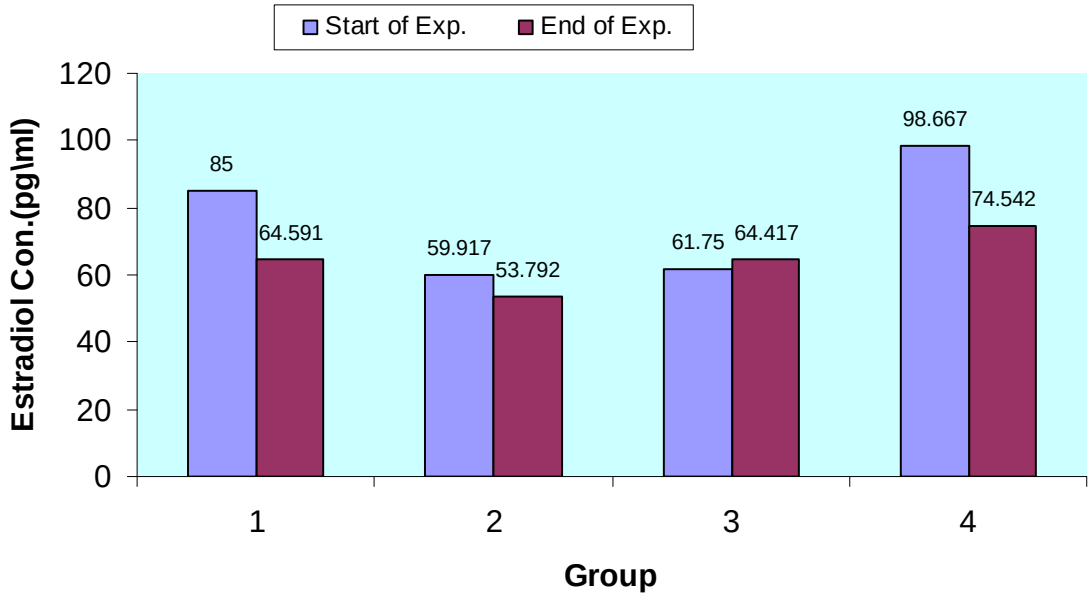


* The result for 1st group (End of Exp.) from 11 ewes

Figure 13: Glycerol as supplement energy effect on LH

5.2.2.3 ESTRADIOL HORMONE.

Differences between groups were insignificantly (P<0.05) for estradiol hormone in two stages (start and at the end) of experiment. Estradiol concentration at the start of experiment was highest in control group (98.667 pg\ml) than others, but in 1st (glycerol 30%) group was higher (85.000 pg\ml) than 3rd (glycerol 10%) group (61.750 pg\ml) and 2nd (glycerol 20%) group (59.917 pg\ml) which is lowest value. At the end of experiment, estradiol concentration highest value was recorded in control group (74.542 pg\ml) and lowest value was registered in 2nd (glycerol 20% group (53.792 pg\ml). Estradiol concentration groups at the end of experiment (pregnancy period) 1st (64.591 pg\ml), 2nd (53.792 pg\ml), and control (74.542 pg\ml) were decreased comparison with respective groups at the start of experiment, conversely than 3rd group which increased (64.417 pg\ml) at the end of experiment (Figure 14).

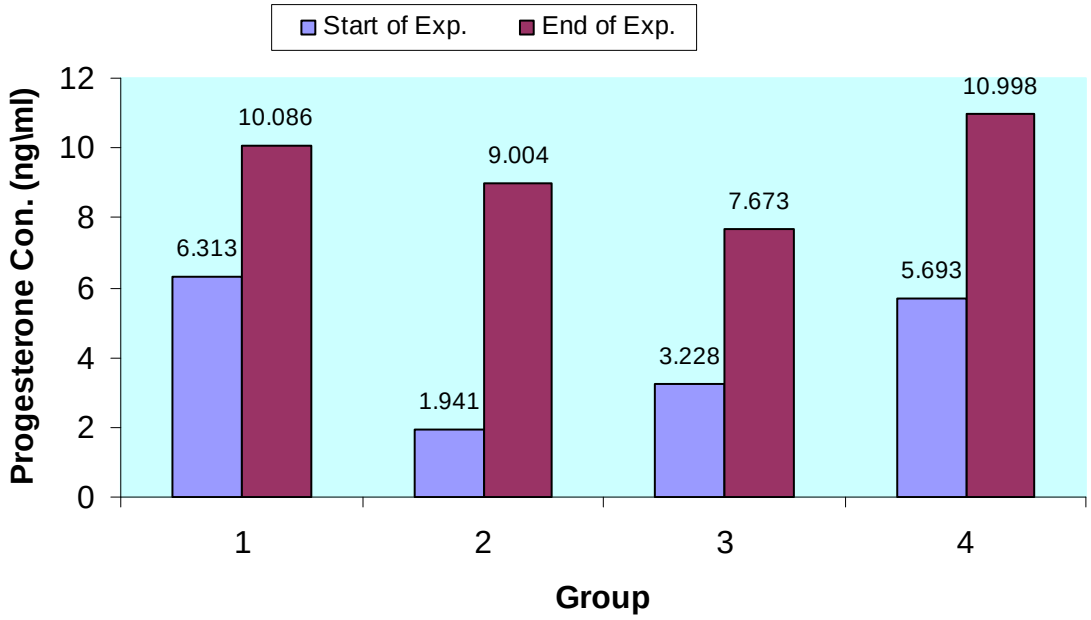


* The result for 1st group (End of Exp.) from 11 ewes

Figure 14: Glycerol as supplement energy effect on Estradiol hormone

5.2.2.4 PROGESTERONE HORMONE.

Progesterone affected by glycerol (30%) insignificantly ($P<0.05$) at the start of experiment that recorded highest value in 1st group (6.313 ng\ml) and lowest value was recorded in 2nd (glycerol 20%) group (1.941 ng\ml), but at the end of experiment we observed control group was increased (10.998 ng\ml) insignificantly ($P<0.05$), while 3rd (glycerol 10%) group was decreased (7.673 ng\ml). The comparison between two stages of experiment, we observed at the end of experiment (pregnancy period) all groups were higher than respective groups at the start of experiment (Figure 15).

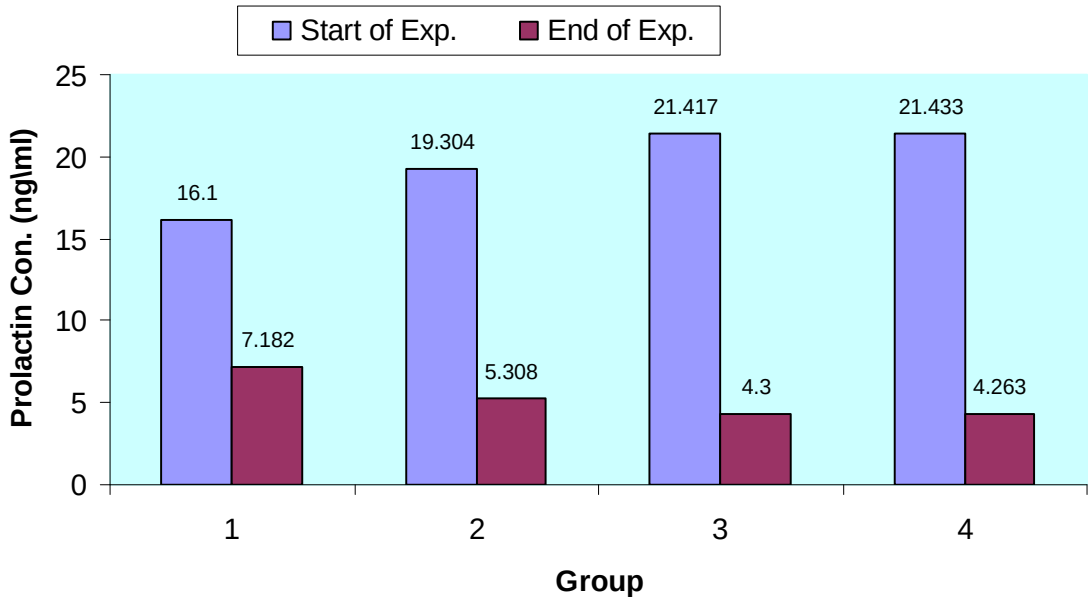


* The result for 1st group (End of Exp.) from 11 ewes

Figure 15: Glycerol as supplement energy effect on Progesterone hormone

5.2.2.5 PROLACTIN HORMONE.

According the results there were insignificant ($P<0.05$) differences between groups for prolactin in two stages (start and at the end) of experiment that increased in 4th group (21.433 ng\ml) and decreased in 1st (glycerol 30%) group (16.100 ng\ml) at the start of experiment. At the end of experiment (pregnancy period), prolactin concentration was increased in 1st (glycerol 30%) group (7.182 ng\ml) and decreased in control group (4.263 ng\ml). Prolactin concentrations in all groups at the start of experiment were higher than respective groups at the end of experiment (pregnancy period) (Figure 16).



* The result for 1st group (End of Exp.) from 11 ewes

Figure 16: Glycerol as supplement energy effect on Prolactin hormone

5.2.3 GLYCEROL EFFECT AS SUPPLEMENT ENERGY ON BIOCHEMICAL PARAMETERS.

5.2.3.1 ALKALINE PHOSPHATASE (ALP).

According the results there were significant ($P<0.05$) differences between groups for ALP in two stages (at the start and the end) of experiment. In the 1st group (30% glycerol) was higher (1.826±0.605) than other groups which was decreased in 2nd (glycerol 20%) group (1.219±0.463) at the start of experiment. At the end of experiment (pregnancy period), also 1st group was higher (1.591±0.592) than other groups that registered lower value in 2nd group. Generally ALP concentration were decreased at the end of experiment (pregnancy period) comparison with groups of the start of experiment respectively (Table 21).

5.2.3.2 ALANINE TRANSAMINASE (ALT).

At the ALT results, there were insignificant (P<0.05) differences between groups in both stages (start and end) of experiment that recorded a higher value in 1st group (30% glycerol) (22.267±5.536) and lower value was recorded in 2nd group (glycerol 20%) (21.408±4.675) at the start of experiment. ALT concentration at the end of experiment (pregnancy period) was increased in 3rd group (10% glycerol) (24.413±5.045) and decreased in 2nd (glycerol 10%) group (21.485±5.561). The comparison between two stages of experiment, the results showed ALT concentrations in all groups at the end of experiment (pregnancy period) were higher than respective groups at the start of experiment (Table 21).

5.2.3.3 ASPARTATE TRANSAMINASE (AST).

AST increased insignificantly (P<0.05) in 1st group (30% glycerol) in both stages (start and end) of experiment respectively (79.057±8.333) (83.465±11.425), and decreased in; 2nd group (71.350±10.886) at the start of experiment and 3rd group (73.291±12.841) at the end of experiment. Also results showed, at the end of experiment the AST concentrations were higher than respective groups at the start of experiment (Table 21).

Glycerol as supplement energy effect on serum enzymes

Table 21

Group	ALP (µkat/l)		ALT (U/l)		AST (U/l)	
	Start of Exp.	End of Exp.	Start of Exp.	End of Exp.	Start of Exp.	End of Exp.
Group 1	1.826±0.605 b	1.591±0.592 b *	22.267±5.536	24.265±8.030 *	79.057±8.333	83.465±11.425 *
Group 2	1.219±0.463 a	0.983±0.397 a	21.408±4.675	21.485±5.561	71.350±10.886	74.219±11.117
Group 3	1.579±0.662 ab	1.270±0.658 ab	21.887±3.886	24.413±5.045	72.852±10.189	73.291±12.841
Group 4 Control	1.305±0.577 a	1.178±0.399 ab	21.431±4.207	22.901±5.707	78.733±12.864	81.313±18.464
Range	0.10- 2.60		15- 44		49- 123	

[a b] Means values with different superscripts within a column differ significantly (P<0.05)

* Result from 11 ewes

5.2.3.4 TOTAL PROTEIN.

There were insignificant (P<0.05) differences between groups for total protein in two stages of experiment that recorded a higher values in 2nd group (20% glycerol) in both stages of experiment respectively (67.799±6.562) (72.880±9.493) and decreased in 3rd group (glycerol 10%) also in two stages of experiment respectively (64.326±5.148) (64.614±11.035). Total protein concentrations were increased at the end of experiment (pregnancy period) groups comparison with respective groups at the start of experiment (Table 22).

5.2.3.5 UREA.

Urea results indicate significantly (P<0.05) differences between groups at the start and end of experiment was observed, that recorded a lower values in; 2nd group (20% glycerol) at the start of experiment (4.749±1.034) and 1st group (30% glycerol) at the end of experiment (4.941±0.802). While urea concentrations in 2nd, 3rd and 4th groups were increased at the end of experiment (pregnancy period) comparison with respective groups at the start of experiment, conversely than 1st group which decreased at the end of experiment when compared with the same group at the start of experiment (Table 22).

5.2.3.6 GLUCOSE.

There were insignificant (P<0.05) differences between groups for glucose in both stages (start and at the end) of experiment. This observed lower value in 2nd group (glycerol 20%) (3.069±0.602) and higher value was recorded in control group (3.538±0.764) at the start of experiment. Whereas at the end of experiment (pregnancy period) a higher value was found in 2nd group (glycerol 20%) (3.293±0.633) and a lower value was registered in 1st group (glycerol 30%) (3.040±0.844). Glucose concentrations in 1st, 3rd and control groups at the end of experiment (pregnancy period) were lower than respective groups at the start of experiment, conversely than 2nd group which increased at pregnancy period (Table 22).

5.2.3.7 TOTAL CHOLESTEROL.

Total cholesterol results indicate insignificantly ($P<0.05$) differences between groups were observed in both stages (start and end) of the experiment, which increased in 3rd group (10% glycerol) (1.165±0.220) and decreased in control group (1.137±0.299) at the start of experiment. At the end of experiment (pregnancy period) 1st group (glycerol 30%) was recorded a higher value (1.982±0.522) and lower value was registered also in control group (1.704±0.364). We were observed cholesterol rise at the end of experiment (pregnancy period) groups comparison with respective groups at the start of experiment (Table 22).

5.2.3.8 TRIGLYCERIDE.

Triglyceride increased insignificantly ($P<0.05$) in 3rd group (glycerol 10%) at the both stages (start and at the end) of experiment respectively (0.240±0.104) (0.262±0.117) and decreased in 2nd group (glycerol 20%) also at the two stages of experiment respectively (0.222±0.105) (0.243±0.132) .Whereas triglyceride concentration were increased at the end of experiment groups when compared with respective groups at the start of experiment (Table 22).

Glycerol as supplement energy effect on blood biochemical properties

Table 22

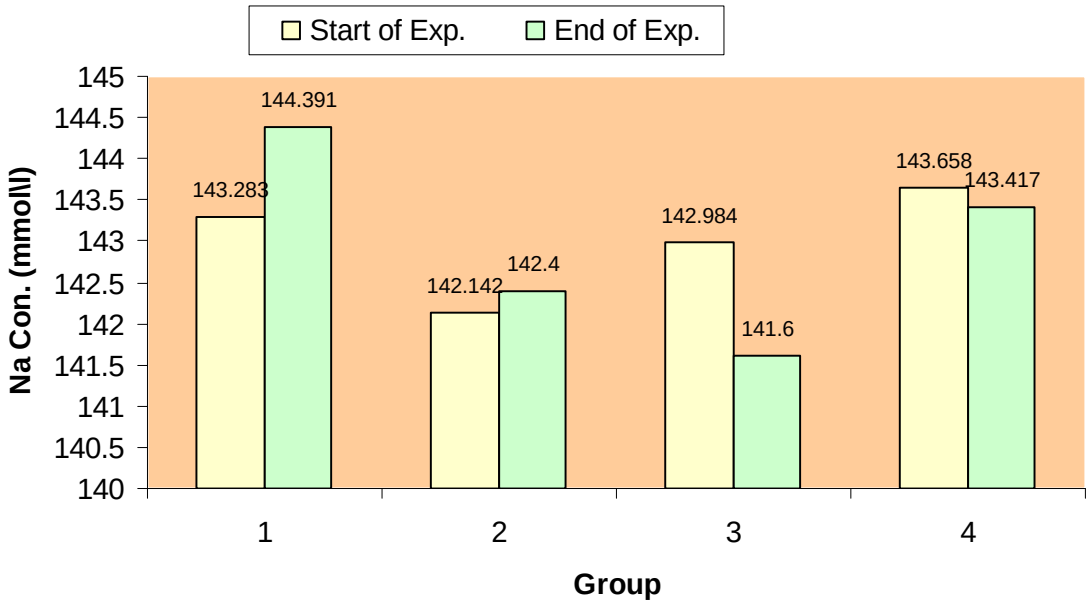
Group	Total Protein (g/l)		Urea (mmol/l)		Glucose(mmol/l)		Cholesterol(mmol/l)		Triglyceride(mmol/l)	
	Start of Exp.	End of Exp.	Start of Exp.	End of Exp.	Start of Exp.	End of Exp.	Start of Exp.	End of Exp.	Start of Exp.	End of Exp.
Group 1	67.518±5.960	70.980±11.493 *	5.098±1.034 a	4.941±0.802 * a	3.131±0.621	3.040±0.844 *	1.151±0.273	1.982±0.522 *	0.239±0.080	0.256±0.125 *
Group 2	67.799±6.562	72.880±9.493	4.749±1.034 a	4.964±1.135 a	3.069±0.602	3.293±0.633	1.145±0.192	1.815±0.478	0.222±0.105	0.243±0.132
Group 3	64.326±5.148	64.614±11.035	4.952±0.792 a	5.091±1.461 a	3.272±0.886	3.089±0.627	1.165±0.220	1.973±0.359	0.240±0.104	0.262±0.117
Group4, Control	64.789±5.730	70.237±9.597	6.081±1.394 b	6.522±1.979 b	3.538±0.764	3.198±0.536	1.137±0.299	1.704±0.364	0.235±0.069	0.249±0.111
Normal range	62- 86		2.8- 8.0		2.4- 4.5		1.1- 2.3		0.15- 0.50	

[a b] Means values with different superscripts within a column differ significantly (P<0.05)

***** Result from 11 ewes

5.2.3.9 SODIUM (Na).

The results showed there were insignificant (P<0.05) differences between groups for sodium that decreased in 2nd group (glycerol 20%) (142.142 mmol\l) and increased in control group (143.658 mmol\l) at the start of experiment. At the end of experiment (pregnancy period), 1st group (glycerol 30%) was increased (144.391 mmol\l) then decreased in 3rd group (glycerol 10%) (141.600 mmol\l). Na concentrations at the end of experiment (pregnancy period) were increased in 1st and 2nd groups, while decreased in 3rd and 4th groups when compared with respective groups at the beginning of experiment (Figure 17).



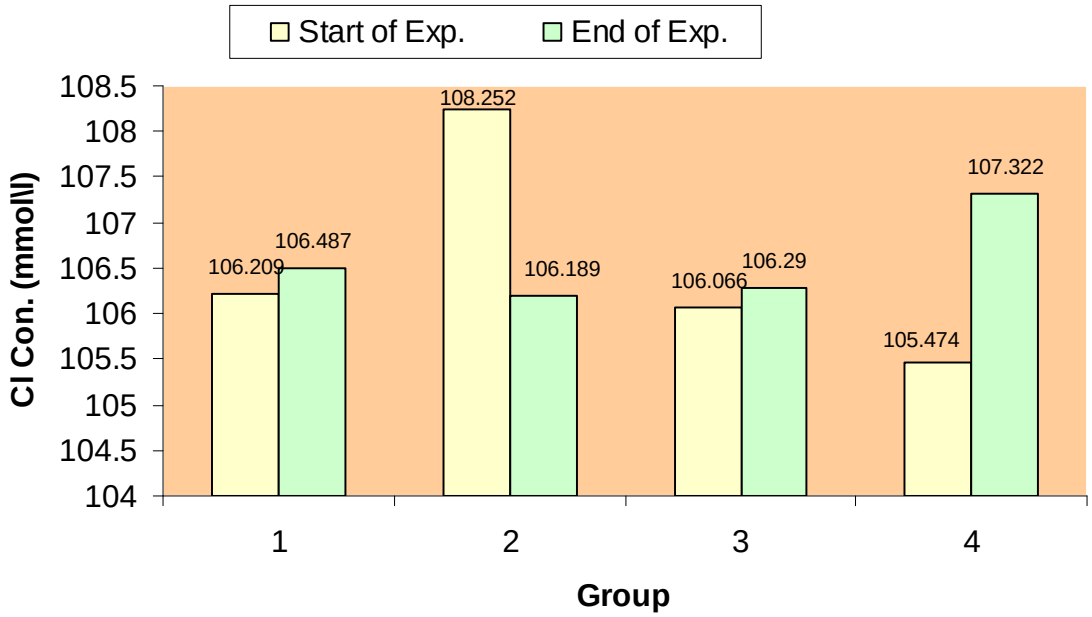
* The result for 1st group (End of Exp.) from 11 ewes
Normal range (130- 155)

Figure17: Glycerol as supplement energy effect on serum Sodium

5.2.3.10 CHLORINE (Cl).

Cl results indicate insignificantly (P<0.05) differences between groups at the start and the end of experiment that recorded highest value 2nd group (glycerol 20%) (108.252 mmol\l) and lowest value was registered in control group (105.474 mmol\l) at the start of

experiment. While control group was increased (107.322 mmol\l) and decreased in 2nd group (106.189 mmol\l) at the end of experiment (pregnancy period). However Cl concentrations in 1st, 3rd and control groups were increased at the end of experiment (pregnancy period) comparison with respective groups at the start of experiment, conversely than 2nd group which decreased in pregnancy period (Figure 18).



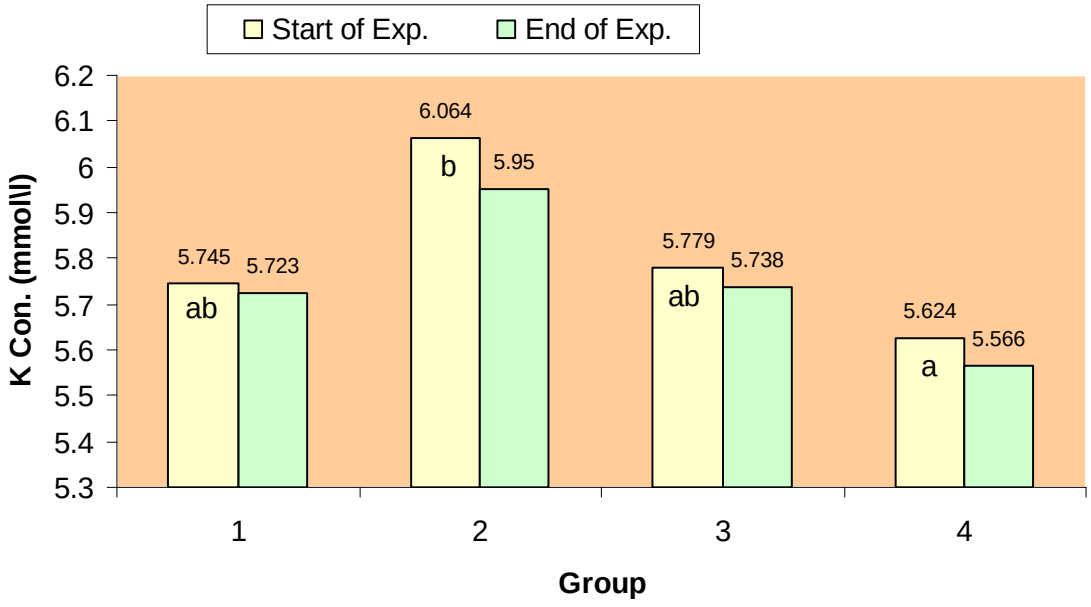
* The result for 1st group (End of Exp.) from 11 ewes
Normal range (90- 115)

Figure18: Glycerol as supplement energy effect on serum Chloride

5.2.3.11 POTASSIUM (K).

There were significant (P<0.05) differences between groups for K at the start of experiment that recorded 2nd group (glycerol 20%) a higher value (6.064 mmol\l) and lower value was recorded in control group (5.624 mmol\l).Whereas at the end of experiment (pregnancy period) were observed insignificant (P<0.05) differences between groups for K that increase in 2nd group (5.950 mmol\l) and decreased also in control group (5.566 mmol\l). While K concentrations at the end of experiment (pregnancy period) were

decreased in all groups when compared with respective groups at the start of experiment (Figure 19).



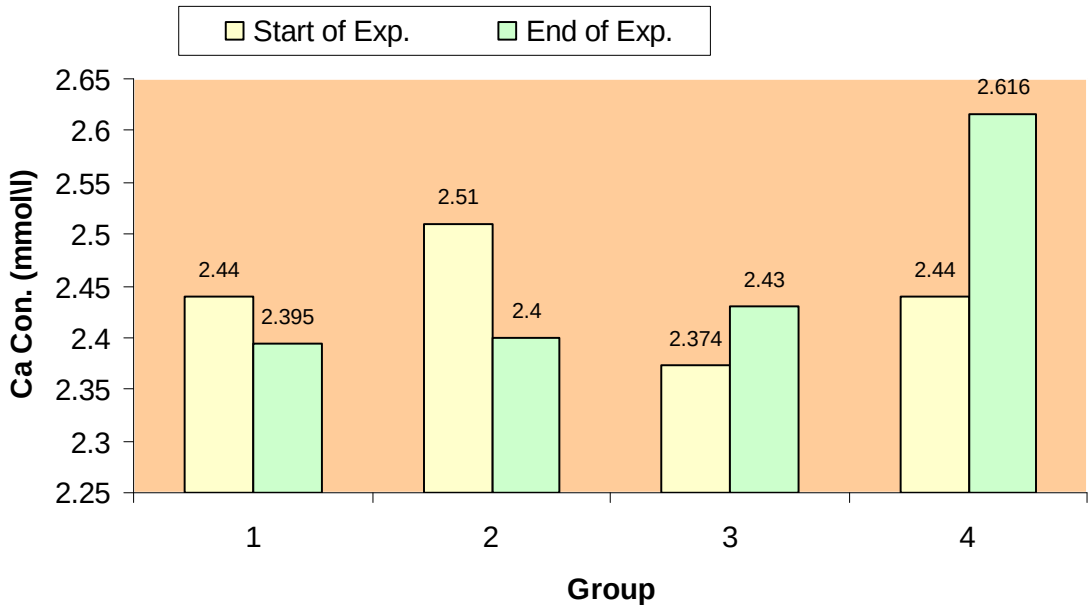
* The result for 1st group (End of Exp.) from 11 ewes

a b Means values with different superscripts within a column differ significantly (P<0.05)
Normal range (3.9- 6.0)

Figure19: Glycerol as supplement energy effect on serum Potassium

5.2.3.12 CALCIUM (Ca).

At the Ca results, there were insignificant (P<0.05) differences between groups in both stages (start and the end) of experiment, that recorded highest values in 2nd group (2.510 mmol\l) and lowest value was recorded in 3rd group (2.374 mmol\l) at the start of experiment. At the end of experiment (pregnancy period), Ca concentration was decreased 1st group (2.395 mmol\l) then increased in control group (2.616 mmol\l). Ca concentrations were decreased in 1st and 2nd groups, conversely than 3rd and control groups were increased at the end of experiment (pregnancy period) comparison with respective groups at the start of experiment (Figure 20).

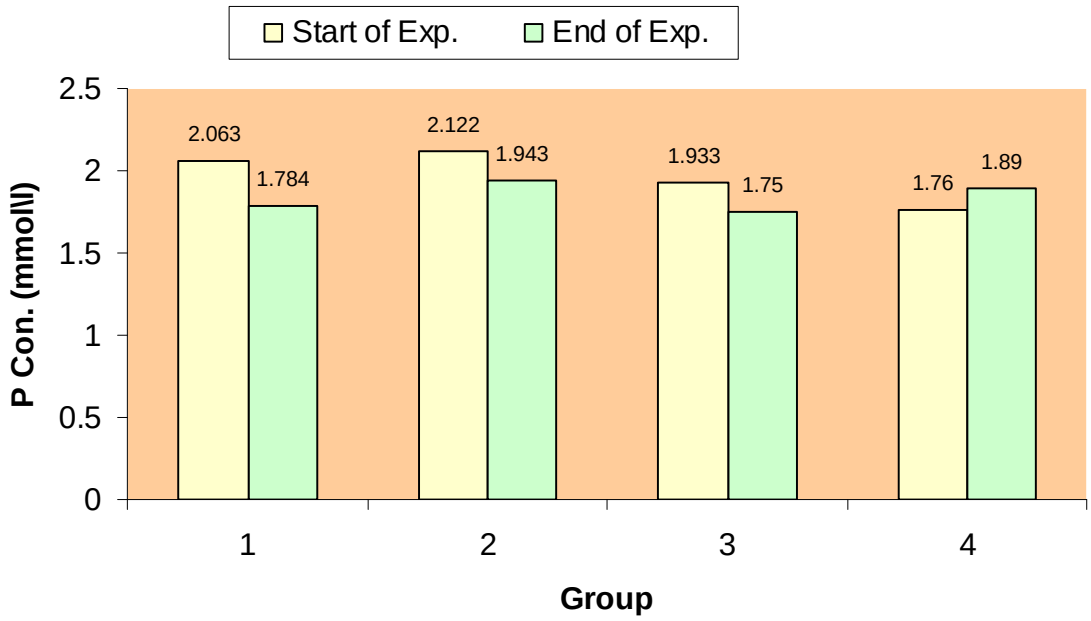


* The result for 1st group (End of Exp.) from 11 ewes
Normal range (2.26- 3.00)

Figure 20: Glycerol as supplement energy effect on serum Calcium

5.2.3.13 PHOSPHOR (P).

Insignificant (P<0.05) differences between groups were observed for Phosphor in two stages (start and at the end) of experiment that recorded 2nd group a higher value (2.122 mmol\l) and lower value was recorded in control group (1.760 mmol\l) at the start of experiment. Also in the 2nd group, P concentration was higher (1.943 mmol\l) but in the 3rd group was lower (1.750 mmol\l) at the end of experiment (pregnancy period). The comparison between two stages of experiment, we observed that Phosphor concentrations at the end of experiment (pregnancy period) were decreased in all groups (except 4th group which increased) than respective groups at the start of experiment (Figure 21).

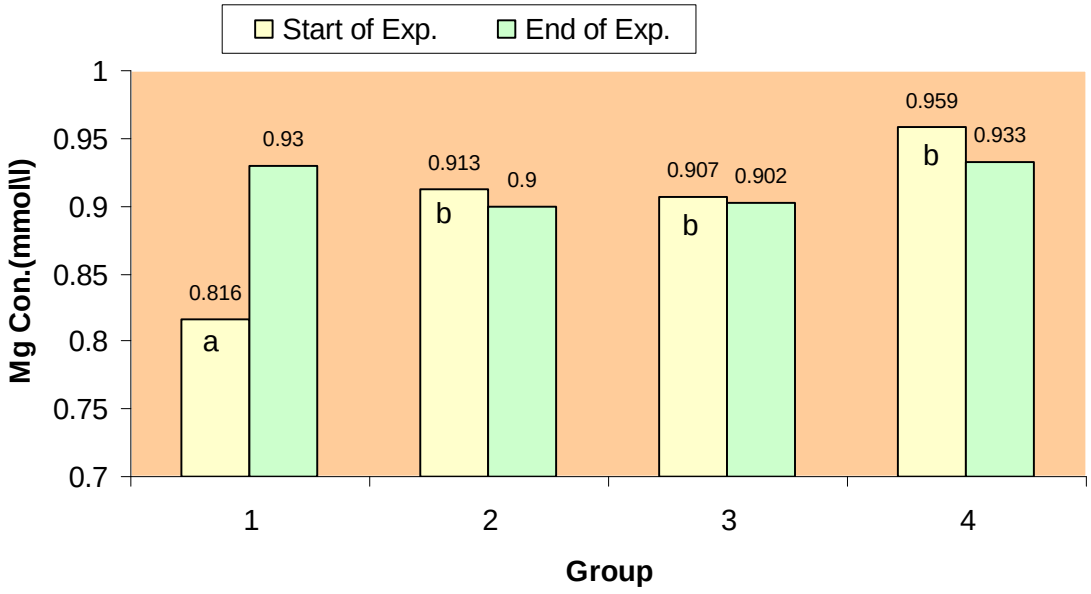


* The result for 1st group (End of Exp.) from 11 ewes
Normal range (1.60- 2.40)

Figure 21: Glycerol as supplement energy effect on serum Phosphor

5.2.3.14 MAGNESIUM (Mg).

Mg results indicate significantly (P<0.05) differences between groups at the start of experiment that recorded lowest value in 1st group (glycerol 30%) (0.816 mmol\l) and highest value was recorded in control group (0.959 mmol\l). However Mg concentration at the end of experiment (pregnancy period) was decreased in 2nd group (0.900 mmol\l) and increased in control group (0.933 mmol\l) insignificantly (P<0.05). Mg concentrations in 2nd, 3rd and control groups were lower, conversely than 1st group (it was higher) at the end of experiment (pregnancy period) when compared with respective groups at the start of experiment (Figure 22).



* The result for 1st group (End of Exp.) from 11 ewes

[a b] Means values with different superscripts within a column differ significantly (P<0.05)
Normal range (0.80- 1.15)

Figure 22: Glycerol as supplement energy effect on serum Magnesium

5.2.4 GLYCEROL EFFECT AS SUPPLEMENT ENERGY ON HAEMATOLOGICAL PARAMETERS.

5.2.4.1 WHITE BLOOD CELLS (WBC).

Glycerol 30% effected on white blood cells insignificantly (P<0.05) in both stages (start and at the end) of experiment that observed in 1st group at the start of experiment was lower (3.778±1.176) and at the end of experiment was higher (3.965±1.379), and WBC in control group was increased (5.123±1.701) at the start of experiment then decreased at the end of experiment (2.776±1.227). Generally WBC groups (except 1st group) at the end of experiment (pregnancy period) were depressed than respective groups at the start of experiment (Table 23).

5.2.4.2 LYMPHOCYTE (LY).

Lymphocyte results indicate there were insignificant (P<0.05) differences between groups at the start of experiment that registered 3rd group (glycerol 10%) a higher value (67.392±6.343) while in control group was depressed (63.354±7.447). Whereas at the end of experiment (pregnancy period), the differences between groups were significantly (P<0.05) which recorded a higher value in control group (67.592±7.637) and lower value was founded in 1st group (57.818±8.240). LY results showed 1st, 2nd and 3rd groups were lower conversely than control group which increased at the end of experiment when compared with respective groups at the start of experiment (Table 23).

5.2.4.3 GRANULOCYTE (GR).

Insignificant (P<0.05) differences between groups were founded for GR at the start of experiment which decreased in 3rd group (32.117±6.334) and increased in control group (36.145±7.447), but significantly (P<0.05) differences between groups were observed at the end of experiment (pregnancy period) which increased in 1st group (glycerol 30%) (41.682±8.240) then decreased in control group (31.908±7.637). Generally all GR groups were increased (except control group which decreased) at the end of experiment when compared with groups at the start of experiment respectively (Table 23).

5.2.4.4 RED BLOOD CELLS (RBC).

There were insignificant (P<0.05) differences between groups for RBC in two stages (start and at the end) of experiment. RBC affected by glycerol 30% which increased in the start of experiment and at the end of experiment respectively (12.265±0.654) (11.705±0.764), whereas 3rd group was depressed (11.526±0.877) at the start of experiment and 2nd group was lower (11.353±0.801) at the end of experiment. All RBC groups at the end of experiment (pregnancy period) were decreased comparison with respective groups at the start of experiment (Table 23).

5.2.4.5 HEMOGLOBIN (HGB).

Insignificant (P<0.05) differences between groups were founded for HGB in both stages (start and at the end) of experiment. HGB concentration was higher in 2nd group

(130.000±11.410) and lower value was recorded in 3rd group (121.917±12.887) at the start of experiment. Whereas at the end of experiment (pregnancy period) 3rd group was higher (131.583±12.802) and in 2nd group was decreased (123.500±14.613). Two HGB groups (1st and 2nd) were decreased and two others (3rd and control) were increased at the end of experiment (pregnancy period) when compared with respective groups at start of experiment (Table 23).

5.2.4.6 HEMATOCRITE (HCT).

Differences between groups for HCT were significantly (P<0.05) at the start of experiment which increased in 2nd group (38.816±2.906) and decreased in 3rd group (35.844±3.792). While at the end of experiment, the differences between groups were insignificantly (P<0.05) that increased in control group (33.465±2.652) then decreased in 1st group (32.661±3.007). The comparison between two stages of experiment, we observed HCT concentrations in all trial groups at the end of experiment (pregnancy period) were lower than respective groups at the start of experiment (Table 23).

Glycerol as supplement energy effect on haematological parameters

Table 23

		Group 1	Group 2	Group 3	Group 4, Control	Normal range
WBC (x10 ^{9}\L)	Start of Exp.	3.778 ± 1.176	4.118 ± 1.986	3.786 ± 1.615	5.123 ± 1.701	5.1 - 11.1
	End of Exp.	3.965 ± 1.379 *	3.296 ± 1.696	3.124 ± 1.811	2.776 ± 1.227	
LY (%)	Start of Exp.	64.967 ± 7.782	66.675 ± 7.508	67.392 ± 6.343	63.354 ± 7.447	32 - 76
	End of Exp.	57.818 ± 8.240 * a	62.542 ± 6.361 ab	60.200 ± 10.443 a	67.592 ± 7.637 b	
GR (%)	Start of Exp.	34.533 ± 7.782	32.833 ± 7.499	32.117 ± 6.334	36.145 ± 7.447	23 - 69
	End of Exp.	41.682 ± 8.240 * b	36.958 ± 6.361 ab	39.300 ± 10.443 b	31.908 ± 7.637 a	
RBC (x10 ^{12}\L)	Start of Exp.	12.265 ± 0.654	12.046 ± 0.888	11.526 ± 0.877	11.880 ± 0.950	5.2 - 11.2
	End of Exp.	11.705 ± 0.764 *	11.353 ± 0.801	11.359 ± 0.999	11.428 ± 0.827	
HGB (g\L)	Start of Exp.	127.833 ± 9.252	130.000 ± 11.410	121.917 ± 12.887	128.083 ± 12.781	90 - 140
	End of Exp.	124.910 ± 13.612 *	123.500 ± 14.613	131.583 ± 12.802	129.417 ± 9.940	
HCT (%)	Start of Exp.	38.268 ± 2.193 ab	38.816 ± 2.906 b	35.844 ± 3.792 a	38.047 ± 4.076 ab	30 - 40
	End of Exp.	32.661 ± 3.007 *	32.877 ± 3.559	33.380 ± 2.473	33.465 ± 2.652	

[a b] Means values with different superscripts within a row differ significantly (P<0.05)

* Result from 11 ewes

6. DISCUSSION:

6.1 GLYCEROL EFFECT ON REPRODUCTION CHARACTERSTICS.

The most important factor determining the success of sheep production is its reproductive efficiency, which is the net biological accomplishment of all reproductive activities i.e. puberty, estrus, ovulation, fertilization, implantation, gestation and successful lambing. It reflects the fecundity, fertility and prolificacy of an adult animal (Khan et al., 2000).

6.1.1 ESESTRUS LENGTH.

An important component of assisted reproduction in sheep is assisted estrus that represents a complex system of methodological procedures and measures for reaching the fertile estrus in a certain period for productive use according to requirements of a breeder and market for the main products of the given flock (Maraček et al., 2009).

According glycerol using as a energy replacement and as a energy supplement in ewes nutrition, results showed it was decreased in two trials of our study, there were significant ($P<0.05$) differences between groups that recorded glycerol 20% group as replacement in (trial I) a lower values than 1st and 4th groups, when glycerol used as supplement in ewes diet, we observed glycerol 30% group caused to decreased estrus length significantly ($P<0.05$). This variation (in estrus length) between glycerol and another groups may be due to high estrogen levels in the blood produced following that is founded in trial (I) and induced luteolysis and stimulation of follicular growth in the ovary by FSH which they were higher in trial (II). It appears that high levels of serum estrogen concentrations are be responsible for a prolonged duration of the estrus period observed in some groups in this study which is agreement with (Ahmed et al.,1998; Dogan and Nur, 2006; Qoja, 2009).

6.1.2 GRAVIDITY.

Insignificant ($P<0.05$) differences between groups for gravidity which founded in 1st and 3rd groups (glycerol 20 % as replacement) were higher than 2nd and control groups in trial (I). Whereas in trial (II) three glycerol groups were higher than control group insignificantly ($P<0.05$). It means for glycerol active role on gravidity when using as

energy replacement or supplement in ewes diet. El-hag et al., (1998) reported that supplemented ewes had higher (P<0.05) conception rate compared to the control un supplemented ones in Sudan. The pregnancy rate or overall conception rate (percentage of total goats diagnosed pregnant) was significantly (P < 0.05) greater for goats in the two treatment groups (3% or 5% fat supplement) than those of the control group (0% fat supplement) (Titi and Awad, 2007). While Aguilar-Perez et al., (2009) observed that supplementation with a calcium soap of long chain fatty acids is not effective for improving pregnancy rate in low-merit grazing cows in the tropics. Ewes fed glycerol had 100% pregnancy rate, improved gravidity was a direct effect of improved conception rate. Such results are similar to those of De Fries et al., (1998); Hegazy et al., (1999); and Hess et al., (2005) who found that adding supplemental fat improved pregnancy rates in ewes and beef cows. Gravidity increase per ewes maybe attribute to elevated progesterone which Skalan et al., (1991) concluded that higher pregnancy rates following fat supplementation were due to elevated progesterone levels after treatment.

6.1.3 PREGNANCY LENGTH.

Pregnancy length affected by glycerol insignificantly (P<0.05) in two trials of study, which registered a highest value in 3rd group (glycerol 20% as replacement) than another respective groups 1st, 2nd and 4th group in trial (I). This results is similar to that found Titi and Awad,(2007) which goats fed supplemental fat 5% than other groups (3% fat supplement and 0% fat supplement) but significantly(P<0.05). Also similar that reported by Lammoglia et al., (1996) who found that beef cows fed high supplemental fat had longer gestation lengths compared to non-fed cows. The same study supported the hypothesis that fat supplementation influences circulating steroid hormone concentrations before parturition. Baguma-Nibasheka et al., (1999) reported a positive correlation between prolonged intake of a high-fat diet in late pregnancy and gestation length and birth weight, possibly due to the alteration of the balance between stimulatory and inhibitory prostaglandins in the parturition process. It is possible that decreased estradiol-17β concentrations in one side and the decreased progesterone clearance rate and its conversion to estrogens at the end of gestation in cows receiving supplemental fat might have altered other endocrine profiles, therefore prolonging gestation (Lammoglia et al., 1996; Baguma-

Nibasheka et al., 1999). Decreased estrogen production would result in a decrease in PGE2 synthesis and thus in delay or prevention of the switch of the myometrial contractility pattern to labor-type contractions (Baguma-Nibasheka et al., 1999).

While in trial (II) which used glycerol as energy supplement, higher values was recorded in 3rd group (glycerol 10%) then followed 2nd group (glycerol 20%) and 1st group (glycerol 30%) respectively compared to lower value which recorded in control group, that's clear which glycerol 30% as supplement shorter period for pregnancy length. This results agree with Munoz et al., (2008) that observed gestation length was shorter ($P<0.05$) for ewes offered diet H-EP (high of predicted metabolisable energy in early pregnancy 200%) than L-EP (low of predicted metabolisable energy in early pregnancy 60%), with M-EP (medium of predicted metabolisable energy in early pregnancy100%) ewes. In other side Nilsson, (1976) reported that gestation length increased with increasing age of the ewe up to 4 years of age. And singles born lambs had longer gestation period than twin born lambs (Musa et al., 2005). This result was in agreement with Newton and Edelsten, (1976) and supporting the results which obtained in our study. Bradford et al., (1972) indicated that the genotype of the fetus is the major determinant of length of gestation period. In sheep male lambs are carried longer than females, single lambs longer than twins (Khan et al., 2000).

6.1.4 NUMBER OF LAMBS.

In first trial (when used glycerol 20% as replacement), number of lambs affected by glycerol insignificantly ($P<0.05$), which recorded in 3rd group a higher value than other respective groups in 1st , 2nd and control group. While in trial (II) (during using glycerol as supplement), Number of lambs affected by glycerol 30% significantly ($P<0.05$) which registered a higher number in 1st group than 2nd group then 3rd group and lower number was recorded in 4th (control) group. This result agrees with Qoja, (2009) that recorded higher number of lambs in ewes group fed glycerol in diet. Khan et al., (2003) was recorded significant ($P<0.05$) increase in the total number of lambs born to hCG-treated ewes compared to saline treated controls. This result can be attributed to the ovulation rate specially twining rate that found in group fed glycerol in the diet, because feeding supplementary fat sources was associated with increasing basal LH concentration and pluse

amplitude and increasing diameter of the largest follicle that increasing twining rate (De Fries et al.,1998). Our result disagree with Lassoued et al., (2004) that founded more lambs in ewes received the moderate nutritional level than ewes on a higher nutritional plan, but these differences were not significant also. And supplementary rolled barley feeding during mating period in Norduz ewes did not affect lamb number per lambed and mated ewes (Demirl et al., 2004).Number of lambs born per ewe was not affected by plan of nutrition in early or mid-pregnancy (Munoz et al., 2008). Furthermore Titi and Awad, (2007) showed that total number of kids per experimental group was similar between control goats and those of the 3% supplemental fat. Whereas Emsen and Yaprak, (2006) mentioned that nutritional flushing (400 g barley ewe/day and 1.5 kg dried grass hay ewe/day) was resulted a higher number of lambs born in Awassi and Red Karaman ewes.

The comparison between obtained results from total ewes in Žirany farm that fed animals without glycerol in year 2008 and used glycerol 15% as supplement in feed ration in year 2009, the number of lambs was 243 from 187 ewes (129.9%) in year 2008, while in year 2009 was 279 from 193ewes (144.5%), and this results it means for glycerol effect on number of lambs that increased when used glycerol 15% as supplement in ewes feed. This results in agreement and supporting our results.

6.1.5 SINGLE LAMBING.

The effect of type of birth and age of dam more important during the pre weaning period than at later ages (Sawalha et al., 2007). Single lambing showed insignificantly ($P<0.05$) differences between groups were observed in trial (I) when used glycerol 20% as replacement, which decreased in glycerol group compared to other groups in 1^{st} , 2^{nd} and control group. Whereas in trial (II) during glycerol using as supplement with different levels in diet, we observed single lambing was decreased in 1^{st} group (glycerol 30%) and increased in 3^{rd} group (glycerol 10%) insignificantly($P<0.05$). The first trial results agree with Qoja, (2009) which used glycerol as energy replacement in ewes nutrition. These maybe attributed to effect due age or physiological maturity and the number of pregnancies (Coop, 1962) or to the important interactions between genotype and level of nutrition are involved in reproductive performance of ewes (Lassoued et al., 2004).

6.1.6 TWINS LAMBING.

Twins lambing results indicated there were insignificantly (P<0.05) differences between groups, while glycerol 20% as replacement (3rd group) was recorded highest value than other groups equally in trial (I). Furthermore when glycerol using as supplement at the trial (II), differences between groups for twins lambing were also insignificantly (P<0.05) and a highest values was recorded in 1st group (glycerol 30%) then in 2nd group (glycerol 20%) and then in 3rd group (glycerol 10%) while in 4th (control) group recorded a lowest value. These results in agreement with authors Khan et al.,(2003), Timurkan and Yildiz, (2005), Qoja, (2009). This maybe attributed to glycerol effect according different levels that lead to increase steroid hormones concentration then increase the number of follicles and therefore raised the twins lambing. Al-Haboby et al., (1999) was obtained significant improvements in twinning percentage when ewes supplemented prior to and during mating with feed block. Insignificantly-higher twins lambing in group content glycerol according with different levels in diet disagree with Titi and Awad, (2007) results that recorded higher (P<0.05) twining rate for goats fed the 3% supplemental fat and 0% supplemental fat in diet compared to those fed the 5% diet. Demiral et al., (2004) found supplementary feeding in addition to grazing during breeding season not affect twinning rate in Norduz ewes. While Musa et al., (2005) found twinning rate was higher in winter lambing than in wet summer lambing in West African sheep. Feeding supplementary fat sources was associated with increasing basal LH concentration and pulse amplitude and increasing diameter of the largest follicle (De Fries et al., 1998). Mechanisms might have resulted in the increased twining rate that caused to increased twins lambing which observed in this study.

The comparison between obtained results from total ewes in žirany farm that fed animals without glycerol in year (2008) and used glycerol 15% as supplement in feed ration in year (2009), twinning rate was 30% in year 2008, while in year 2009 was 45%, and this results it means for glycerol effect on twinning rate that increased when used glycerol 15% as supplement in ewes feed. This results in agreement and supporting our results.

6.1.7 TRIPLET LAMBING.

Triplet lambing only we founded in trial (II) when used glycerol as supplement according with different levels. Results indicate insignificantly (P<0.05) differences

between groups, whereas 1st (glycerol 30%) group was highest value than other groups equally which not founded similar situation for other groups. The reason could be attributed to increase steroid hormone concentrations that caused to increased ovulation rate and increase the number of follicles. Such an increase in ovulation rate might have resulted from a faster rate of maturation and/or ovulation of immature follicles (Kinser et al., 1983). Khan et al., (2003) indicated to increases the proportion of animals producing triplets collectively resulted in a significant ($P<0.05$) increase in the total number of lambs born to hCG-treated ewes compared to saline treated controls.

6.1.8 MALE LAMBS.

Insignificantly ($P<0.05$) differences between groups were founded for male lambs in two trials, whereas a highest value was recorded in control group, but glycerol group was higher than 1st group in trial which used glycerol as replacement (trial, I). While in trial (II) when used glycerol as supplement, a higher number of male lambs was recorded in 3rd (glycerol 10%) group and a lower numbers was recorded in 2nd (glycerol 20%) group. Whereas 1st group (glycerol 30%) was highest than 2nd and control groups. In my theory, this cause maybe attributed to genetic factor for breed type. Khan et al., (2000) indicated that total lambs born to 201 ewes were 469, including 221 males. Thus, the sex ratio of male to female was 47.12:52.88 in Rambouillet X Kaghani crossbred sheep. While Ishaque,(1972) observe the ratios of male to female as 54:46, 50:50, 51:49 for 3270, 2862 and 2281 berths, respectively.

The comparison between obtained results from total ewes in žirany farm that fed animals without glycerol in year 2008 and used glycerol 15% as supplement in feed ration in year 2009, number of male lambs was 124 from total 243 lambs (51%) in year 2008, while in year 2009 was 131 from total 279 lambs (47%), and this results it means for glycerol effect on number of male lambs that increased when used glycerol 15% as supplement in ewes feed. This results in agreement and supporting our results.

6.1.9 FEMALE LAMBS.

There were significantly ($P<0.05$) differences between groups for female lambs, that showed the results in 1st and glycerol (20% as replacement) groups were higher than

2nd and control groups respectively in trial (I). Whereas in trial (II) when glycerol using as supplement, the results indicate for differences between groups were insignificantly (P<0.05), in 2nd group (glycerol 20%) was higher than 1st group (glycerol 30%). Possible to predict during these results that glycerol 20% addition to ewes diet as (replacement or supplement) increase female lambs born. And could be attributed to important interaction between genotype and level of nutrition are involved in reproduction performance of ewes (Lassoued et al., 2004), or to energy supplementations of animals that improved fertility (Smith and Somade, 1994). Khan et al., (2000) indicated that total lambs born to 201 ewes were 469, including 248 females. Thus, the sex ratio of female to male was 52.88: 47.12 in Rambouillet X Kaghani crossbred sheep.

The comparison between obtained results from total ewes in Žirany farm that fed animals without glycerol in year 2008 and used glycerol 15% as supplement in feed ration in year 2009, number of female lambs was 119 from total 243 lambs (49%) in year 2008, while in year 2009 was 148 from total 279 lambs (53%), and this results it means for glycerol effect on number of female lambs that increased when used glycerol 15% as supplement in ewes feed. This results in agreement and supporting our results.

6.1.10 MALE LAMBS WEIGHT.

The lamb birth weight is one of the most important factors influencing pre-weaning growth in young animals, since lambs heavier at birth grow faster than lightweight lambs (Notter and McClaugherty, 1991; Susic et al., 2005).

Variance analyses of data for male lambs weight revealed no significant (P<0.05) differences between groups for two trials of study. When used glycerol 20% as replacement (trial I), the results showed that control group was highest than other groups. Whereas the weight of male lambs in glycerol (3rd) group was higher than 1st and 2nd groups respectively. While in trial (II) during glycerol using as supplement, results showed 3rd group (glycerol 10%) was highest than other groups. Whereas the weight of male lambs in 1st group (glycerol 30%) was higher than control and 2nd (glycerol 20%) groups respectively. This result in agreement with Qoja, (2009) study which obtained on the same results when used glycerol in ewes nutrition. Titi and Awad, (2007) indicated that goats fed 5% supplemental fat had a heavier male birth weight than the 3% supplemental fat and

control groups. While Demirel et al., (2004) observed supplementary feeding in addition to grazing during breeding season in Norduz ewes did not affect male lamb weights. The differences in birth weight in the current study may have been partly due to differences in ambient environment and maternal pre-natal effects during gestation (Yilmaz et al., 2007) or maybe attributed to genetic factor for ewes. The energy balance of dams during the last trimester is a greater influence on birth weight than actual BW or BCS of dams at parturition (Lake et al., 2005).

6.1.11 FEMALE LAMBS WEIGHT.

In two trials of study, the results showed there were significant ($P<0.05$) differences between groups that recorded 1st group and 3rd group (glycerol 20%) highest values respectively than 2nd and control groups when used glycerol as replacement (trial I). While in trial (II), glycerol using as supplement, the results showed 1st group (glycerol 30%) was higher than 2nd group (glycerol 20%) then 3rd group (glycerol 10%) and then lower value was recorded in 4th (control) group respectively. Titi and Awad, (2007) mentioned that goats fed 5% supplemental fat had a heavier female birth weight than the 3% supplemental fat and control groups. While Demirel et al., (2004) observed supplementary feeding in addition to grazing during breeding season in Norduz ewes did not affect female lamb weights. Lammoglia et al., (1996) suggested that maternal variation in circulating concentrations of estrogen in late-pregnant cows could be influenced by placental size and/or function that are reflected in the birth weight of the calf. They reported that dietary fat before calving may influence birth weight through changes in circulating steroid hormone concentrations at the end of pregnancy. And could be the potential beneficial effect of feeding glycerol on ewe performance may be more pronounced in dams with a poor body condition.

6.1.12 TOTAL LAMBS WEIGHT.

Maternal undernutrition during pregnancy has negative effects on fetal development and offspring health. However, the effect of maternal undernutrition about the time of conception on neonatal outcome is not clear (Smith et al., 2010).

Differences between groups was significantly (P<0.05) for total lambs weight in two trials of study. A higher value was recorded in 3rd group (glycerol 20%) than other groups which observed when used glycerol as replacement in trial (I). Whereas in trial (II), a highest value was founded in 1st group (glycerol 30%) than other groups respectively. That is mean total lambs weight affected by glycerol (20% as replacement and 30% as supplement). This result in agreement with Qoja, (2009) when glycerol used in ewes diet, and agree with Titi and Awad, (2007) result that increased birth weight in goats fed 5% supplemental fat. Opposite to our results, lambs weight was not affect in many studies (Lammoglia et al., 1996; Bottger et al., 2002). While Petit and Berthiaume, (2006) indicated that birth weight of calves was similar among treatments when beef cows fed different sources of fat during gestation. No differences were detected for calf birth weight when cows supplemented fat at parturition and postpartum period (Lake et al., 2005). Differences in weight could be a result of greater utilization efficiency of dietary energy as feeding supplemental fat has been reported not only to increase energy density but also to improve energetic efficiency (Lammoglia et al., 2000). Sabra and Hassan, (2008) mentioned in his present study that flushing of Barki ewes with the present mixture for one month prebreeding improve the reproductive performance indicated by the increase lamb birth weight significantly. While Demirel et al., (2004) observed supplementary feeding in addition to grazing during breeding season in Norduz ewes did not affect lamb weights.

6.1.13 FERTILITY.

One important economic constraint of the sheep industry is the seasonal nature of ewe fertility (Notter, 2002).

There were insignificant (P<0.05) differences between groups for fertility percentage in two trials of study, which recorded a higher values in glycerol (20% as replacement) and 1st groups than 2nd and control groups in trial (I). Whereas in trial (II) when glycerol using as supplement, we were observed in first three groups with equal values were bigger than 4th (control) group. Fertility was higher insignificantly (P<0.05) in animals fed glycerol in diet. This result agree with Qoja,(2009) which indicated ewes fertility was increased in group fed glycerol 20% as energy replacement in nutrition. Musa et al., (2005) stated that fertility rate was (78.69%) in winter lambing of West African sheep in Sudan that not agree

with our result. Also Hoedemaker et al., (2004) observed that no differences between control group and group supplemented propylene glycol in dairy cows fertility. Maybe the reason attributed to fertility increase progressively as the type of birth of lambs increased from single to triplets (Anisworth and Shrestha, 1987). Khan et al., (2003) mentioned that a single injection of hCG given at the onset of a synchronized estrus has a potential to improve the fertility of ewe lambs. There are many different reports which indicate that the type of energy fed has a significant influence on dairy cow fertility. It has been reported that feeding diets which result in a relatively high supply of glucogenic nutrients (mostly ruminal propionate and glucose) results in less mobilization of adipose tissue as measured by blood metabolites (Rizos et al., 2004). In addition, there are some reports that such diets improve fertility in dairy cattle (Gong et al., 2002). Many experiments have reported beneficial effects of supplemental fat on fertility indices in dairy cattle (Staples et al., 1998). Intensification of the milking sheep reproduction means an increase in the fertility by a rise of the mean natality through the increase in multi-fetus (especially twins). This can be reached by increasing the whole life fertility of ewes (Ptaszynska, 2002). This high fertility can be related to the higher ovarian activity and could be attributed in high energy metabolism from glycerol.

6.1.14 PROLIFICACY.

Insignificantly ($P<0.05$) differences between groups for prolificacy was founded in two trials of study. In trial (I) glycerol group was registered a great percentage when used glycerol 20% as replacement in ewes diet. While In trial (II), using of glycerol as supplemental in diet, 1st group (glycerol 30%) was greater than other respective groups, then 2nd group (glycerol 20%) was higher than 3rd (glycerol 10%) and 4th groups, contrary to control group that registered a lower percentage. Prolificacy was higher in animals fed glycerol in diet. Our results agree with Hegazy et al., (1999) that found prolificacy rate was improved when ewes fed different types of supplemental fat during late and early lactation. De Fries et al., (1998) reported that dairy cows fed supplemented fat had larger and more mature preovulatory follicles than non-fed cows. And also stated that this effect might be beneficial due to the presence of substantial increase in the number of potential ovulatory follicles. Dietary fat may enhance follicular development through metabolites and

metabolic hormones that act to influence GnRH secretion (De Fries et al., 1998) and thereby improve number of lambs and twining rate. Jainudeen and Hafez, (1987) also reported prolific sheep respond better to hormonal treatment for the purpose of estrus synchronization than less prolific breeds. Lassoued et al., (2004) was concluded from his study that prolificacy is a major differentiating factor between genotypes in his study, in the high prolific D'Man breed, higher levels of nutrition prior to and during mating were associated with improved reproductive performance in accordance with the literature reported for several sheep breeds. While Santos et al., (2009) observed no difference in the rate of proliferation among the treatment groups when ewes given nutritional supplements to enhance energy levels in spring mating.

6.1.15 FECUNDITY.

Increasing the productivity of the sheep by increasing fecundity could be considered important option for increasing the reproduction efficacy (Emsen and Yaprak, 2006).

There were significant ($P<0.05$) differences between groups for fecundity in two trials of study. In first trial, when used glycerol as replacement, 3rd group (glycerol 20%) was greater than other groups. While in second trial, when used glycerol as supplement, 1st (glycerol 30%) and 2nd (glycerol 20%) groups were registered equal highest percentage than respective 3rd group (glycerol 10%) and 4th (control) group which they registered lower percentage respectively. This result agree with Qoja,(2009) which indicated ewes fecundity was increased in group fed glycerol 20% as energy replacement in nutrition. Musa et al., (2005) stated that fertility rate was (78.69%) in winter lambing of West African sheep in Sudan that not agree with our result. Also Hoedemaker et al., (2004) observed that no differences between control group and group supplemented propylene glycol in dairy cows fertility. Maybe the reason attributed to fertility increase progressively as the type of birth of lambs increased from single to triplets (Anisworth and Shrestha, 1987). Pitta et al., (2004) observed the increase in the reproductive rate of ewes that grazed on full access to willow was multifactorial. The most significant reason was due to the increase in fecundity in ewe grazed willow fodder blocks during drought.

6.2 GLYCEROL EFFECT ON HORMONES.

There is a little information which found on variations in hormones in direct relation to nutrition effect especially with energy.

6.2.1 FOLLICLE STIMULATING HORMONE (FSH).

Glycerol effected FSH insignificantly ($P<0.05$) in two trials of study that decreased at the three stages (start, estrus and at the end) of first experiment respectively when used glycerol as replacement in diet. FSH concentration in 1st and 4th groups were decreased in the estrus phase then increased at the end of experiment respectively, while FSH concentrations in 2nd and 3rd groups were increased gradually in the estrus and then at the end of experiment respectively. While in trial (II) when glycerol using as supplement in diet, 1st group (glycerol 30%) was increased in two stages (at the start and end) of experiment respectively and followed 3rd group (glycerol 10%) in two respective stages (at the start and end) of experiment that showed greater than 2nd and control groups. Generally, FSH concentrations in first trial groups were increased in pregnancy period (at the end of experiment) when compared with beginning and in estrus of experiment groups, but in trial (II), FSH concentrations were decreased in groups of pregnancy period (at the end of experiment) compared to respective groups at the start of experiment.

Many factors are implicated in the differential modulation of FSH secretion (Price, 1991). Steroids affect their response via receptors and alterations in the concentrations of these receptors may be a means by which the pituitary alters its sensitivity to the effects of circulating estradiol, accounting for discordant patterns in the secretion of FSH. While Bartlewski et al., (2000) observed there was no positive correlation between the number of the pulse frequency of follicle stimulating hormone (FSH) and the plasma concentrations of progesterone during the luteal phase in ewes. Suganuma et al., (2007) results indicate that progesterone has no physiological role in the regulation of FSH secretion or its own secretion from the corpus luteum as a local stimulator during the early luteal phase of the estrus cycle. Whereas Kotsampasi et al., (2009) indicated to maternal undernutrition during the first month of pregnancy resulted in increased pituitary sensitivity to GnRH in terms of FSH response along and increased number of small follicles in the ovary, while during mid to late gestation resulted in a reduction of large corpora lutea in female sheep offspring.

Nutritional influences on reproduction may be linked through variations in the IGF-I system which secreted from the liver or present in other reproductive tissues (Roberts et al., 2001). Minegishi et al., (2000) found that expression of the FSH-receptor was enhanced when IGF-I was added to granulose cell culture medium along with FSH. Kiyma et al., (2004) was found the serum concentrations of FSH did not differ but magnitudes of FSH surges were lower in fasted than in control ewes. The effects of nutrition on the blood concentrations of FSH are very problematic and no consistent picture has emerged despite a large number of published papers on the subject. There are a number of reasons for this lack of clarity. First, the effect of nutrition on FSH remains subject to negative feedback regulation and any nutritional stimulation of FSH concentrations would in theory, be quickly corrected by the negative feedback homeostasis. Second, we have no clear picture of the rapidity of negative feedback correction following nutritional stimulation of FSH. The effect of nutrition on FSH is likely to be rapid, transient and with a response in the normal range of blood concentrations. Bearing this in mind, it is not surprising that the effect of nutrition on FSH concentration remain unclear and difficult to demonstrate (Scaramuzzi et al., 2006).

6.2.2 LUTEINIZING HORMONE (LH).

There were insignificant ($P<0.05$) differences between groups in two stages (start and estrus phase) of experiment, and significantly ($P<0.05$) differences between groups at the end of experiment was observed for LH concentration when used glycerol as replacement in trial (I) which decreased in 3rd group at the estrus phase, LH concentration in respective groups 1st, 2nd and 3rd were increased in estrus phase then decreased at the end of experiment (pregnancy period). Furthermore, in trial (II) when glycerol used as supplemental in diet, there were insignificant ($P<0.05$) differences between groups in two stages (start and at the end) of experiment which observed for LH concentration that decreased in 3rd group (glycerol 10%) and increased in control group, but 1st group (glycerol 30%) was higher than two glycerol groups (2nd and 3rd). Conversely than at the end of experiment (pregnancy period) which observed 3rd group (glycerol 10%) was recorded a higher value then 2nd group (glycerol 20%), but in 1st (glycerol 30%) group LH concentration was decreased. LH concentration groups at the end of experiment (pregnancy

period) were decreased comparison with respective groups at the start of experiment. Kotsampasi et al., (2009) reported that mean plasma LH levels did not differ between groups when evaluated the effect of maternal undernutrition in female sheep offspring. Several studies reported the energy level has been related to variations in LH secretion (Grimard et al., 1995; Lucy et al., 1992; Perry et al., 1991; Schillo, 1992). The type of diet had no significant effect on basal LH concentration in beef cows fed many supply energy (Khireddine et al., 1998; Ponsart et al., 2000). While Butler et al., (2006) found that mean LH was not different between control and propylene glycol cows. Kiyma et al., (2004) was found serum concentration of LH did not differ between control and fasted ewes. Energy provided by fat supplementation increases LH secretion in animals that consume less energy than required (Sklan et al., 1994). Secretion of LH from the pituitary and follicular growth in ruminant are regulated partially by the energy status of the animal. Supplemental fat is included frequently in rations to increase energy concentration in the diet, which could result in an increase in energy intake and improve the energy status of the cow (Harrison et al., 1995). Energy provided by fat supplementation increases LH secretion in animals that consume less energy than required (Sklan et al., 1994). About these reasons we observed LH rise in estrus then decreased at the end of experiment in all groups except in 4^{th} group in trial (I), and depression of LH concentration at the end of two trial (pregnancy period) could be attributed to increase progesterone levels in pregnancy period that caused and depress LH concentrations, because the progesterone act by inhibiting LH secretion from the pituitary (Hafez and Hafez, 2000).

6.2.3 ESTRADIOL HORMONE.

Estradiol hormone affected by glycerol insignificantly ($P<0.05$) in two trials of study. In trial (I) when glycerol used as replacement, estradiol was increased in 3^{rd} group at the two stage of trial (at the start and in estrus phase) of experiment, but unaffected at the end of experiment. Generally, estradiol concentration in 1^{st}, 2^{nd} and 3^{rd} groups were decreased in the estrus phase then increased at the end of experiment (pregnancy period) compared to respective groups at the start of experiment. Conversely than control group, this decreased gradually in estrus phase then at the end of experiment. While in trial (II) when glycerol used as supplement in diet, estradiol concentration in two stages (at the start

and in the end) of experiment were recorded highest values in control group than others, but in 1st group (glycerol 30%) were higher than 3rd group (glycerol 10%) and 2nd group (glycerol 20%) which recorded lowest values. Estradiol concentration groups 1st, 2nd, and control were decreased at the end of experiment (pregnancy period) comparison with respective groups at the start of experiment, conversely than 3rd group which increased at the end of experiment. Sarath et al., (2008) was founded a significantly higher concentration of estradiol on day 4 and 8 in insulin-treated goats as compared to control. Estradiol concentration were higher in pregnant than non pregnant reindeer (Flood et al., 2005) which agreed with our results for trial (I). Depressed concentration of estradiol in estrus phase than at the start of experiment may result from diminished ovarian follicular development caused by suppressed serum concentration on gonadotrophin (Gougeon, 1996). The higher concentration of estradiol for glycerol group at the start of experiment and estrus phase could be attributed to increase the concentration of total cholesterol in granulose cells which leaded to increase estradiol concentration (Wehrman et al., 1991). Decreased concentrations of estradiol for groups (1st, 2nd and control) in pregnancy period for trial (II) may result from diminished ovarian follicular development caused by suppressed serum concentrations of gonadotrophin (Gougeon, 1996). Or it might also be possible that the lower estradiol concentrations could have been caused by the longer gestation length which received high level of glycerol (Lammoglia et al., 1996). Scaramuzzi et al., (2006) reported that positive energy balance effected reproduction hormones which reduced estradiol. Lammoglia et al., (1996) observed in Cows receiving 6.55% dietary fat had a marked decrease in estradiol-17β concentrations before parturition, whereas estradiol-17β concentrations in cows receiving 5.20% dietary fat were increased and estradiol-17β remained constant in control cows, and this in agreement to our results in trial (II). Differences in circulating estradiol concentrations among treatments in this study support the hypothesis that fat supplementation influences circulating steroid hormone concentrations before lambing. Results from this study support those reported by Boyd et al., (1987) that dietary composition affects circulating steroid hormone concentrations before parturition. High nutrition can also increase metabolic clearance rate of steroid hormones such as estradiol which reported by (Roche, 2006).

6.2.4 PREGESTERONE HORMONE.

Progesterone affected by glycerol, significantly ($P<0.05$) at the start of experiment and estrus phase, and insignificantly ($P<0.05$) at the end of experiment in trial (I) when used glycerol as replacement, which registered highest values in 3rd group for three stages of experiment. We observed that glycerol group was decreased in the estrus phase then increased at the end of experiment (pregnancy period) compared to at the start of experiment, and progesterone concentrations in all trial groups were highest at the end of experiment (pregnancy period) comparison with two stages (at the start and estrus phase) of experiment. While in trial (II) when glycerol used as supplemental in diet, progesterone affected by glycerol (30%) insignificantly ($P<0.05$) at the start of experiment that recorded highest value in 1st group and lowest value was recorded in 2nd group (glycerol 20%), but at the end of experiment we observed control group was increased, while 1st group (glycerol 30%) was higher than 2nd group (glycerol 20%) and 3rd group (glycerol 10%) which registered a lower value. The comparison between two stages of experiment, we observed at the end of experiment (pregnancy period) all groups were higher than respective groups at the start of experiment. Several researchers observed fat supplementation effect on progesterone concentration. Staples et al., (1998) reported that cows fed supplemented fat had more active ovaries with higher progesterone concentrations. However, similar progesterone concentrations among treatment groups agreed with the results reported by De Fries et al., (1998) and Moallem et al., (1999) which founded no effect of feeding supplemental fat on progesterone concentration. However, Hightshoe et al., (1991) mentioned that feeding supplemental fat to beef cows improved progesterone concentrations during the luteal phase of the first postpartum estrus cycle. Titi et al., (2008) indicated that progesterone concentration was increased ($P<0.05$) in ewes fed 3% fat addition. The increase level of progesterone during estrus and rise during pregnancy phase were in accordance with Pathak et al., (1992). This high mean progesterone concentration in glycerol group for trial (I) could assume an active corpus luteum (CL) during that stage and might indicate differences in response to the secreted prostaglandin F2α (Titi et al., 2008), or could be attributed to increase the concentration of cholesterol in follicular fluid and related to the fact of granulose cells which increased progesterone concentration in three stages of trial (I), and in agreement with report by (Grummer and Carroll, 1991)

which indicated to dietary fat supplementation in cows consistently increases plasma concentrations of cholesterol (the precursor for the synthesis of progesterone). In other hand Shabankareh et al., (2009) indicated the serum progesterone concentration was significantly higher in double than single ovulating ewes on day 1-16 of the estrus cycle, and the evolution of the corpus luteum is associated with viviparity in mammals and is necessary for the production of progesterone, which is required for growth and maintenance of pregnancy (Powell et al., 2006), and the relatively high incidence of double ovulation in ewes is associated with increasing total luteal volume and high circulating progesterone concentrations (Shabankareh et al., 2009). It's normally to observation high values for progesterone at the end of two trials because in pregnant animals, progesterone concentrations remain elevated throughout gestation (Boscos et al., 2003).We obtained converse results compared to O'Callaghan et al., (2000) that mentioned serum progesterone concentrations were affected by dietary intake, and ewes on high intake had lower progesterone concentrations compared with ewes on lower dietary intakes. These results support the conclusion of other studies that circulating progesterone concentrations are inversely related to feed intake in sheep (Williams and Cumming, 1982; Parr et al., 1987). Maternal undernutrition during two different periods, early (0–30) and early to mid (31–100) days of gestation did not affect the time of the onset of puberty, defined as the first increase in plasma progesterone concentrations (Kotsampasi et al., 2009). Also Kiyma et al., (2004) found that fasting was increased serum concentrations of progesterone in ewes. Whereas Moallem et al., (2007) found the progesterone concentration was higher in the CaLFA group (calcium soaps of long-chain fatty acids) than PGLY group (Propylene glycol) and PrFA group (prilled fat) in cows. Several studies reported fat supplementation increase circulating progesterone concentrations serum and follicular fluid cholesterol concentrations, and the area occupied by lipids in small and large luteal cells (Talavera et al., 1985; Williams, 1989; Hightshoe et al., 1991; Ryan et al., 1992; Hawkins et al., 1995). Titi and Awad, (2007) concluded that concentration of plasma progesterone were not affected by adding supplemental fat in goats .Lammoglia et al., (2000) mentioned the Diet had a significant effect on progesterone concentrations at day 7-10 of the pubertal estrus cycle, and higher progesterone concentrations were found in heifers that received the HF (Hereford dietary fat) diet, which also increased cholesterol concentrations, because

cholesterol is utilized in progesterone synthesis. The flushing of Barki ewes with 200g/ewe daily of a balanced mixture composed of oil bearing grain (58% crushed yellow corn and 41% crushed soy bean) for one month, ewe had higher progesterone level during estrus cycle and allover the gestation period (Sabra and Hassan, 2008). While Aboelmaaty et al., (2008) found the levels of progesterone were high during restriction compared with re-feeding in both groups of goat with no observed significant variation in progesterone between both groups. El-Shahat and Abo-Elmaaty, (2010) were found the serum progesterone concentrations had risen and were significantly higher ($P< 0.05$) for basal diet supplemented with CSFA (calcium salts of long chain fatty acids) than control group.

6.2.5 PROLACTIN HORMONE.

According the results for trial (I) when used glycerol as replacement, there were significant ($P<0.05$) differences between groups for prolactin at the start of experiment that increased in 4^{th} group, while 3^{rd} group (glycerol) was higher than 1^{st} and 2^{nd} groups. There were insignificant ($P<0.05$) differences between groups for prolactin and unaffected by glycerol in estrus and at the end of experiment (pregnancy period). Generally prolactin concentration in respective groups 1^{st}, 2^{nd} and 3^{rd} were increased in estrus phase then decreased at the end of experiment (pregnancy period) compared to respective groups at the start of experiment. Whereas the results of trial (II) when used glycerol as supplemental in diet, there were insignificant ($P<0.05$) differences between groups for prolactin in two stages (start and at the end) of experiment that increased in 4^{th} group, and 3^{rd} group (glycerol 10%) was recorded higher value than 2^{nd} group (glycerol 20%) and 1^{st} group (glycerol 30%) which registered a lowest value at the start of experiment. While at the end of experiment (pregnancy period), prolactin concentration was increased in 1^{st} group (glycerol 30%) and 2^{nd} group (glycerol 20%) was higher than 3^{rd} (glycerol 10%) and control groups which decreased in control group. Prolactin concentrations in all groups at the start of experiment were higher than respective groups at the end of experiment (pregnancy period), this means prolactin concentrations were decrease in pregnancy period. McFadden et al., (1990) was found the mean concentrations of prolactin receptors in crude membranes of mammary parenchymal tissue were increased by feeding the protected fat supplement. Fitzgerald et al., (1981) was observed the changes in basal concentrations of

prolactin in the 6 pregnant and 6 non-pregnant ewes during the first 80 days of pregnancy were low. Kann and Denamur (1974) suggested that the increased secretion of prolactin might be associated with the well known rise in the circulating concentration of estradiol, and but may be related to the changing sensitivity of the hypothalamus to the negative feedback action of estradiol-17β which is known to occur at the end of the breeding season (Karsch et al., 1980). Rhind et al., (1980) was indicated that plane of nutrition had little effect on prolactin levels, and higher prolactin values were recorded during estrus in ewes mated in March or July, and found the mean increases in the concentrations of prolactin during estrus were smaller in lactating than non-lactating ewes. There is evidence that concentrations of prolactin in sheep plasma are elevated during the normal period of sexual inactivity (Pelletier, 1973; Erbet al., 1977) and during lactation (Lamming et al., 1974). Mean concentrations of prolactin receptors in mammary parenchyma were elevated in lambs group fed dietary polyunsaturated fat. Increased availability of receptors may mediate the stimulation of mammary growth observed in lambs fed polyunsaturated fat (McFadden et al., 1990).

6.3 GLYCEROL EFFECT ON BIOCHEMICAL PARAMETERS.

Blood analyses may be used to assess the health and physiological condition of domestic or wild animals, and may provide a precise picture of nutrition, disease, trauma, habitat quality, and other environmental stressors (Perez et al., 2003).

6.3.1 ALKALINE PHOSPHATASE (ALP).

According the results for two trials of study, there were significant ($P<0.05$) differences between groups for ALP. In trial (I) when used glycerol as replacement, the 4th group was greater than other groups, then 3rd group (glycerol) was higher than 2nd and 1st groups respectively in two stages (at the start and at the end) of experiment, At the end of experiment (pregnancy period), ALP concentration for 1st and 2nd groups were increased, while in 3rd (glycerol) and 4th groups were decreased comparison with respective groups at the start of experiment. About ALP concentrations in trial (II) when used glycerol as supplemental in diet, the results showed 1st group (30% glycerol) was higher than other groups, and 2nd group (glycerol 20%) was registered lowest value in two stages (at the start

and the end) of experiment. Generally ALP concentration were decreased at the end of experiment (pregnancy period) compared to respective groups at the start of experiment. Our results agree with Qoja and Šťastný, (2010) which observed similar result for ALP, and disagree with Caldeira et al.,(1999) that found lower ALP serum activity in group fed 30% of theoretical maintenance energy requirements in ewes and the most striking event observed for this variable, also indicated that ALP levels was affected by undernutrtion, but not by overnutrition. And also agree with Klebaniuk et al., (2009) which observed a significantly higher activity of ALP enzyme in cows fed the glucogenic additive (calcium propionate and propylene glycol mixture in loose form), while Sandabe et al.,(2004) mentioned the pregnancy did not induce any change ($P<0.05$) in ALP enzyme in Sahel goats. Although Soch et al., (2008) was found a higher mean values for ALP in pregnant cows for more than 140 days. This results could be indicating that during pregnancy, the liver is not stressed (Sandabe et al., 2004), and was probably caused by lower tares in bone metabolism and in phosphorylation and dephosphorylation reaction (Caldeira et al., 1999). Masek et al., (2007) was found the ALP activity values decreased during the milking period in dairy ewes. Jurajdova and Trcala (1990) was reported no changes in ALP activity during pregnancy. A statistically non-significant increase was reported by Sato et al., (2005) between non-pregnant and pregnant cows. Whereas sheep administrated 200 and 400 viable metacercariae of *fascioliasis gigantica* had significant elevations in serum alkaline phosphatase (Teleb et al., 2007).

6.3.2 ALANINE TRANSAMINASE (ALT).

ALT results indicate significantly ($P<0.05$) differences between groups in two stages (at the start and end) of trial (I) which used glycerol as energy replacement in diet. Higher values were registered in 4^{th} group for two stages (start and end) of experiment. However 3^{rd} group (glycerol) was recorded lower value than other groups in two stages (start and end) of experiment, except than 2^{nd} group at the start of experiment. While ALT in 1^{st} and 3^{rd} groups decreased at the end of experiment (pregnancy period) compared to respective groups at the start of experiment, but in 4^{th} group was increased in the end of experiment in comparison with start of experiment. Whereas in trial (II) when used glycerol as supplemental in diet, there were insignificant ($P<0.05$) differences between groups in

both stages (start and end) of experiment that recorded a higher value in 1st group (30% glycerol) and lower value was recorded in 2nd group (glycerol 20%) at the start of experiment. ALT concentration at the end of experiment (pregnancy period) was increased in 3rd group (10% glycerol) and decreased in 2nd (glycerol 10%) group, and 1st group (glycerol 30%) was higher than 2nd and control groups. The comparison between two stages of experiment, the results showed ALT concentrations in all groups at the end of experiment (pregnancy period) were higher than respective groups at the start of experiment. Generally glycerol groups in pregnancy period was decreased in trial (I) and increased in trial (II) compared to the beginning of experiments. Our results in trial (I) in agreement to reported by Qoja et al., (2010), while our two trials results disagree with; Ozdogan et al.,(2006) that found no statistically significant among treatment groups for ALT activity when used tallow and cotton seed oil in fattening bulls, Klebaniuk et al., (2009) observed the average of ALT activity immediately postpartum was approximately 30% higher than in the dry-off period, and it increased slightly until week 6 of lactation, then the glucogenic supplement did not exert any significant impact on the activity of ALT enzyme in cows. While Cenesiz et al., (2006) observed no differences between groups were determined in activity of serum ALT when supplemented barley with urea molasses mineral blocks in lambs. Masek et al., (2007) was observed the activities of ALT did not differ significantly during the milling period in dairy ewes. The reason, changes that occur in enzyme activity are considered to be as an indicator of the health of an organism, and liver is the terminal controlling organ of the metabolism. By measuring the metabolic activity of liver, determination of functional events could be estimated, and ALT is the specific enzyme of liver which is increased in the plasma by destruction of cell-membrane and cell necroses in acute liver diseases (Cenesiz et al., 2006). Or could be attributed to the physiological status of ewes (pregnancy) influences their hypothalamic-pituitary-adrenal axis activity (Soch et al., 2008). Lactation and late pregnancy are critical periods during the reproductive cycle of the ewe. This is confirmed by significant changes in blood biochemical indices, including enzymatic activity (Borzostowski et al., 1996). Whereas Sobiech et al., (2008) observed throughout lactation, significant ($P \leq 0.05$) changes were observed in both groups of ewes with regard to ALT activity. In ewes suckling singles, the highest activity of this enzyme was recorded on day (28), while the lowest – on day (2) of

lactation. In ewes nursing twins, enhanced activity of ALT was reported on days (28) and (70). Bademkiran et al., (2008) was found insignificant differences in serum ALT activity between the self-sucking group and the control group in dairy cows. Also Mohamed and Abou-Zeina, (2008) was observed insignificant differences among different experimental groups for ALT when goat kids treated sugar beet pulp. In pregnant ewes, the ALT showed early significant increase, from 2nd week of pregnancy (El-Sherif and Assad, 2001). Antunovic et al., (2004) was found statistically higher ALT in the pregnant ewes comparing to the non-pregnant ones and those in lactation. Chiofalo et al., (2009) indicated to the hepatic functionality (ALT) was significant lower in the "Glycol" group during the prepartum as well as the post partum period in dairy goats, which in agreement to our results for trial (I), and this could be related to the propylene glycol (such as glycerol) supplementation that could represent a proper way to rapidly decrease lipid mobilization and excessive formation of ketone bodies, thereby reducing the risk of fatty liver and ketosis (Hoedemaker et al., 2004).

6.3.3 ASPARTATE TRANSAMINASE (AST).

There were significant ($P<0.05$) differences between groups for AST in two stages (at the start and end) of trial (I) which used glycerol as replacement in diet. 4th group was recorded higher values in two stages of experiment, while 3rd group (glycerol) was lower than other groups (except 2nd group) at the start of experiment. At the end of experiment (pregnancy period), AST affected by glycerol significantly ($P<0.05$) that decreased in 3rd group. Generally AST concentration in 1st, 2nd and 3rd groups were higher at the start of experiment than respective groups at the end of experiment (pregnancy period), conversely than control group which increased in pregnancy period. Whereas in trial (II) when used glycerol as supplemental in diet, AST increased insignificantly ($P<0.05$) in 1st group (30% glycerol) in both stages (start and end) of experiment, and decreased in 2nd group at the start of experiment and then 3rd group at the end of experiment. AST concentrations at the end of experiment (pregnancy period) were higher than respective groups at the start of experiment. Our results at trial (I) in agreement to report by Qoja et al., (2010), and with Klebaniuk et al., (2009) which observed a significantly higher activity of AST enzyme in cows fed the glucogenic additive (calcium propionate and propylene glycol mixture in

loose form). Caldeira et al., (1999) found that AST activities remained relatively stable during the 24-h period, with only a small, but significant ($P<0.05$) decrease after the meal in all groups fed different levels of theoretical maintenance energy requirements in ewes. Hoedemaker et al., (2004) also found no significant effect of propylene glycol on the activity of AST in dairy cows. While Mikula et al., (2008) observed no group differences in the AST activity in the transition period. On days 56 and 70 of lactation, the lowest AST activity in all experimental groups was observed with statistically significant differences ($P<0.05$) in comparison to control group. In all groups, mean activity of AST increased from 3rd week before calving to day 14 of lactation and then decreased to day 70 d lactation in dairy cows fed propylene glycol supplementation. Higher AST activities in 1st, 2nd and 3rd groups at the start of experiment for trial (I) could possibly be explained to glucose precursors to counterbalance a phase of energy negative balance in the daily cycle (Caldeira et al., 1999). And higher AST activities at the end of experiment (pregnancy period) groups in trial (II) could be attributed to impairment in some muscle and liver cells due to rapid gluconeogenesis associated with pregnancy, this enzyme was found to be involved in gluconeogenesis(Krebs,1966). Or maybe this transitory increase was not as a pathological condition, but rather as an adjustment of liver cell turnover rate to the increased metabolic demands during late pregnancy (Bostedt, 1974). On other hand, Sandabe et al., (2004) was found pregnancy did not induce any changes in AST, indicating that, during pregnancy the liver is not stressed in Sahal goats. Chiofalo et al., (2009) observed that hepatic functionality (AST) was significant lower in the "Glycol" group during the prepartum as well as the post partum period in dairy goats. While Ozdogan et al., (2006) was found no statistically significant among treatment groups for AST activity when used tallow and cotton seed oil in fattening bulls. Also Mohamed and Abou-Zeina, (2008) was observed insignificant differences among different experimental groups for AST when goat kids treated sugar beet pulp. In pregnant ewes, the AST showed early significant increase, from 2nd week of pregnancy (El-Sherif and Assad, 2001), but in goat, Jana et al., (1991) was found an increase in AST in early pregnancy. Caldeira et al.,(1999) observed lower AST serum activity in group fed 30% of theoretical maintenance energy requirements in ewes and the most striking event observed for this variable, also indicated that AST levels was affected by undernutrtion, but not by overnutrition.

Masek et al., (2007) was observed the activities of AST did not differ significantly during the milking period in dairy ewes. Sobiech et al., (2008) study showed throughout lactation, significant ($P \leq 0.05$) changes were observed in both groups of ewes with regard to AST activity in ewes. While Bademkiran et al., (2008) was found insignificant differences in serum AST activity between the self-sucking group and the control group in dairy cows.

6.3.4 TOTAL PROTEIN.

Total protein results in trial (I) when used glycerol as energy replacement in diet, differences between groups were insignificantly ($P<0.05$) at the start of experiment and significantly ($P<0.05$) at the end of experiment, which recorded 4th group higher value than other groups and 3rd group (glycerol) was higher than 2nd group in both stages (start and end) of experiment. While the results indicate that total protein in 1st and 4th groups at the end of experiment was increased than same groups at the start of experiment respectively, while in 2nd and 3rd groups at the end of experiment (pregnancy period) was decreased compared to respective groups at the start of experiment. Whereas trial (II) when glycerol used as supplemental in diet, the results indicate there were insignificant ($P<0.05$) differences between groups for total protein in two stages of experiment that recorded a higher values in 2nd group (20% glycerol) than other groups in both stages of experiment and decreased in 3rd group (glycerol 10%) also in two stages of experiment respectively. Total protein concentrations were increased at the end of experiment (pregnancy period) groups comparison with respective groups at the start of experiment. Our result in trial (I) is in agreement to Qoja et al., (2010) results, also agree with Ozdogan et al., (2006) that observed differences for serum concentration of total protein among treatment groups was not statistically significant when diets containing tallow and cotton seed oil in fattening bulls. Our result for total protein at the start of trial (I) maybe considered that there was no obvious effect resulting from feeding because these values were within the normal range (Altintas and Fidanci, 1993) .While Caldeira et al., (1999) found significant variations ($P<0.05$) during the period around the meal when fed ewes three different levels of feed. While our results in trial (II), total protein was increased in pregnancy period which agree (not statistically) with Piccione et al., (2009) that showed serum total protein was higher significantly during three stages of pregnancy (early, mid and late) compared to dioestrus in

ewes. Also agree with Qoja and Šťastný, (2010) which observed similar result for total protein. However, in Oetzel et al., (1988) study a significant effect was not reached, probably because of a low difference in protein content in diet or a short period of adaptation to experimental diet. Relative to protein metabolism, Antunovic et al., (2002) observed a blood protein concentration during the late stages of gestation was decrease in sheep, it's possible the utilization of amino acids for protein synthesis in the fetal muscles. A decrease in serum total protein level was recorded on day 150 of gestation, compared to other stages of gestation (60^{th} and 100^{th} day) in Akkaraman ewes (Balikci et al., 2007). El-Sherif and Assad (2001) found plasma protein was increased in 6^{th} week of pregnancy in Barki ewes. While Sandabe et al., (2004) indicated protein concentration remained unchanged between two groups (pregnant and non pregnant goats). Flushing of Barki ewes with 200g/ewe daily of a balanced mixture composed of 58% crushed yellow corn and 41% crushed soy bean for one month was caused increase in plasma total protein significantly (Sabra and Hassan, 2008). Total protein rise in pregnancy period of trial (II) can be interpreted by the facts that the fetus synthesizes all its proteins from the mother, and absolute growth of the fetus increases exponentially reaching a maximum, especially in muscles during late gestation (Jainudeen and Hafez, 1994).

6.3.5 UREA.

There were insignificant ($P<0.05$) differences between groups for urea in both stages (at the start and the end) of trial (I) when used glycerol as replacement, a higher value for urea was recorded at the start of experiment in 3^{rd} group (glycerol). While at the end of experiment, 3^{rd} group (glycerol) was higher than control group and lower than 1^{st} and 2^{nd} groups. From the results we observe that urea concentration in 1^{st} and 4^{th} groups were increased and in 2^{nd} and 3^{rd} groups were decreased at the end of experiment (pregnancy period) comparison with respective groups at the start of experiment, and it means clearly that glycerol group was decreased in pregnancy period. Whereas in trial (II) when used glycerol as supplemental in diet, significantly ($P<0.05$) differences between groups at the start and end of experiment were observed for urea, which recorded a lower values in; 2^{nd} group (20% glycerol) at the start of experiment and 1^{st} group (30% glycerol) at the end of experiment. While urea concentrations in 2^{nd}, 3^{rd} and 4^{th} groups were

increased at the end of experiment (pregnancy period) comparison with respective groups at the start of experiment, conversely than 1st group which decreased at the end of experiment compared to same group at the start of experiment. Our result in trial (I) in agreement to Qoja et al., (2010) when used glycerol in ewes diet. While Sandabe et al., (2004) was found pregnancy did not induce any changes in urea, and indicating that, during pregnancy the kidney is not stressed. Chiofalo, (2000) observed the addition of 80 g ewe/d of propylene glycol into the diet decreased plasma urea ($P<0.01$) and this finding could be attributed to a higher gluconegenesis. Rekik et al., (2007) mentioned to using polyethylene glycol as supplement in ewe diet did not affect plasma urea. Chiofalo et al., (2009) observed during using propylene glycol supplementation in dairy goats, urea concentrations in glycol groups were lower than control groups in two periods (prepartum and postpartum). Urea value was significantly lower in group used 2.5% cotton seed oil than group used 2.5% tallow and without fat group in fattening bulls (Ozdogan et al., 2006). Piccione et al., (2009) showed a significant decrease for urea at the end of lactation and in dry period compared to pregnancy period in ewes. The reason for those highest values for urea in groups could be the increased cortisol level affecting the catabolism of protein in the body (Silanikove, 2000). Balikci et al., (2007) mentioned that plasma urea levels were lower at 150 days of pregnancy and 45 days postpartum than at 60 days of gestation. El-Sherif and Assad (2001) reported plasma urea to start increasing in pregnant ewes from 10th week of pregnancy, reaching a maximum level at parturition. Shetaewi and Daghash (1994) found the level of serum urea during pregnancy to slightly exceed that of early lactation but it was highest at 55 days of lactation. Contrary, Brozostowski et al. (1996) observed an increase in urea level during early pregnancy. Sandabe et al., (2004) indicated urea concentration unchanged between pregnant and non pregnant goats. In ruminants, amino acids are not normally catabolised and are used for synthesis of milk proteins, and subsequently urea production in the body falls and plasma urea concentration decreases. Chiofalo, (2000) observed that urea concentrations were higher in control group than each from low level group (80 g/ewe/day) and high level group (160 g/ewe/day) respectively during using propylene glycol in prepartum period in dairy ewes which supporting our result at the end (pregnancy period) of trial (II). On the other hand the reason for high urea concentration in glycerol groups or

in pregnant ewes could be related to either high protein metabolism during pregnancy or nutritional management.

6.3.6 GLUCOSE.

There were insignificant ($P<0.05$) differences between groups for glucose in both trials of study. In trial (I) when used glycerol as replacement, results show 3rd group (glycerol) was registered highest value at the start of experiment and lowest value was recorded at the end of experiment (pregnancy period). Generally glucose in 1st, 3rd and 4th groups was decreased at the end of experiment (pregnancy period) comparison with respective groups at the start of experiment conversely than 2nd group which increased at the end of experiment (pregnancy period).While in trial (II) when used glycerol as supplemental in diet, 3rd group (glycerol 10%) was higher than 1st (glycerol 30%) and 2nd (glycerol 20%) groups, and all of them were lower than control group at the start of experiment. But at the end of experiment (pregnancy period), 2nd group (glycerol (20%) was higher than others, whereas 1st group (glycerol 30%) was registered lower value. Glucose concentrations in 1st, 3rd and control groups at the end of experiment (pregnancy period) were lower than respective groups at the start of experiment, conversely than 2nd group which increased at pregnancy period. Our results in trial (I) in agreement with several studies; Qoja et al., (2010) when used glycerol in ewes diet, while Bodarski et al., (2005) that found glucose concentration was lower in group fed glycerin compared to other groups in dairy cows which is supported our result in trial (I) at the pregnancy period, Mikula et al., (2008) observed that the blood glucose concentration prepartum and postpartum was not significantly affected by glycol supplementation in dairy cows, Firat and Ozpinar (1996) recorded plasma glucose levels to be lower in pregnant than in non-pregnant sheep, DeFrain et al.,(2004) that mentioned no differences were detected for prepartum concentrations of glucose, then feeding glycerol tended to decrease postpartum concentration of glucose plasma of dairy cows, and Balikci et al., (2007) indicated that the serum glucose was lower ($P<0.05$) in twin-bearing than in single bearing sheep on days 100 and 150 of gestation in Akkaraman ewes. While Wang et al., (2009b) found glucose concentration in plasma increased with increasing glycerol supplementation in Holstein dairy cows feed, Chiofalo (2000) observed the addition of propylene glycol increased

plasma glucose concentration in ewes. Our results could be attributed to the first metabolic pathway of glycerin is more effective for ruminants, from rest of glycerin or propionic acids the glucose and could be formed in liver. This way of glycerin changes to glucose maybe a better for ketosis prophylactics (Remond et al., 1993). From the other side a second metabolic pathway of glycerin transformation to butyric acid is more dangerous for ewes, because this volatile fatty acid is a main precursor of betahydroxybutyric acid - ketosis biochemical marker (Remond et al., 1991). Studies in dairy ewes indicate that the supplementation of propylene glycol during the close-up dry period and early lactation improved the metabolic condition, through the increasing of glucose concentrations (Chiofalo et al., 2005). While Chiofalo et al., (2009) found glucose in glycol group was lower than control group in prepartum period in dairy goats. The values of glucose were significantly higher in bulls fed 2.5% tallow in nutrition than other groups (Ozdogan et al., 2006). Mohamed and abou-Zeina,(2008) mentioned there were no significant differences among different experimental groups concerning serum glucose when goat kids treated sugar beet pulp in diet, which supported our results, and could be attributed to this parameter was within the normal range of ewes (Mert,1996). The glucogenic supplement elevated the concentration of glucose in cows (Klebaniuk et al., 2009). Milewski and Sobiech, (2009) was noted that addition of Saccharomyces cerevisiae dried brewer yeast to diets for lactating ewes increase glucose concentration. Pambu-Gollah et al., (2000) concluded that glucose plasma concentrations was sensitive to seasonal changes in nutrient supply, and that they could be of use as indicators of nutritional status in situations in which conventional methods of nutritional assessment. Plasma glucose concentrations were also sensitive to changes in nutrient demand (i.e. altered physiological state), raising the possibility that they could be employed to evaluate the suitability of genotypes with different milk production potentials within a given nutrient environment. Several autheos reported that glucose concentration were higher in pregnancy (El-Sherif and Assad, 2001; Ferat and Ozpinar, 2002; Chiofalo et al., 2005), contrary than other which observed that glucose was lower in pregnancy period (Sandabe et al., 2004; Antunovic et al., 2004; Balikci et al., 2007; Moghaddam and Hassanpour, 2008). The rise of glucose concentrations in different levels of glycerol groups in our study could be explain to indicating that glycerol has an efficacious glucogenic effect that favours the increase of

gluconeogenesis, glycogenolysis, or both, stimulated by catecholamines and glucocorticoids (Studer et al., 1993). Juchem et al., (2004) observed lower glucose concentration in prepartum cows treated with glucoplastic substances. The limited effect of the glycerol on glucose blood level could be explained by a large increase in insulin concentration that maintains plasma glucose (Brockman et al., 1986) or by the concentration of insulin peaking before that of glucose. Chung et al., (2007) mentioned to dry glycerin fed as a top dressing at 250 g/d (corresponding to 162.5 g of food grade glycerol/d) to multiparous Holstein dairy cows from parturition to 3 wk into lactation tended to improve energy availability (higher blood glucose) of cows during the second week of lactation. While Schieck et al., (2010) was found glucose concentrations were not affected by crude glycerol in lactating sows.

6.3.7 TOTAL CHOLESTEROL.

There were insignificant ($P<0.05$) differences between groups for total cholesterol in two trials of study. In trial (I) when used glycerol as replacement, results indicate that total cholesterol affect by glycerol which decreased at 3rd group (glycerol) in both stages (start and at the end) of experiment. Generally, total cholesterol concentration in all groups decreased at the end of experiment (pregnancy period) comparison with respective groups in the start of experiment. Whereas in trial (II) when used glycerol as supplemental in diet, total cholesterol in 3rd group (glycerol 10%) was higher than others, while in 1st group (glycerol 30%) was higher than 2nd group (glycerol 20%) and control groups at the start of experiment. At the end of experiment (pregnancy period), a higher values were recorded in respective groups as follows in 1st group (glycerol 30%), 3rd group (glycerol 10%), 2nd group (glycerol 20%) and lower value was registered in control group. Results indicate that total cholesterol was elevated at the end of experiment (pregnancy period) groups compared to respective groups at the start of experiment. Our results in trial(I) agree with; Qoja et al., (2010) which used glycerol in ewes diet, Chiofalo et al.,(2000) study that observed plasma total cholesterol was lower in propylene glycol groups during the prepartum period in ewes, Piccione et al., (2009) found in total cholesterol was decreased during late gestation in ewes, Mohamed and Abou-Zeina, (2008) reported there were no significant ($P<0.05$) differences among different experimental groups concerning total cholesterol in goats used

sugar beet pulp in dietary supplementation, Chiofalo et al., (2009) which mentioned the glycol group showed lower level for cholesterol in dairy goats, and Mikula et al.,(2008) that founded the mean cholesterol concentration in blood was not affected by the treatment (propylene supplementation) during transition period, and in all groups mean cholesterol concentration decreased before parturition in dairy cows. While trial (II) results is in agreement with; Sandabe et al., (2004) observed the serum cholesterol concentrations in pregnant goats were higher than non pregnant, Balikci et al., (2007) was found increase ($P<0.05$) in serum cholesterol during pregnancy in Akkaraman ewes, and Ozdogan et al., (2006) indicated the value of cholesterol concentration were significantly higher in group fed 2.5% cottonseed when compared with their respective mean concentration in group fed 2.5% tallow and group fed without fat in fattening bulls. Decrease cholesterol concentrations in two stages of trial (I) maybe attributed to result from a temporary reduction of liver capacity (Chiofalo, 2000). Cholesterol increase during pregnancy period in trial (II) may be due to insulin, which plays a direct role in adipose tissue metabolism during pregnancy and its responsiveness is significantly reduced in ewes during late pregnancy (Jainudeen and Hafez, 1994; Schlumbohm et al., 1997). The diminished responsiveness of the target tissue to insulin during late pregnancy predisposes the ewes to increase of cholesterol concentration (Schlumbohm et al., 1997), or maybe the high plasma cholesterol concentrations in the absence of excess dietary energy intake are considered to reflect the capacity of the animal to mobilize body fat reserves (Ruegg et al., 1992). Cenesiz et al., (2006) reperted no changes in concentration of total cholesterol were found among groups of lambs supllemented urea molasses minaral blocks. Bademkiran et al., (2008) noted the level of serum cholesterol was significantly increased in seff- sucking dairy cows. Pambu-Gollah et al., (2000) observed plasma cholesterol concentrations were higher ($P < 0.05$) in lactating goats during months two and three of lactation (July, August) than in nonlactating goats, furthermore, plasma cholesterol concentrations during the winter lactation were also substantially higher than during the summer lactation season. This suggests that body adipose tissue reserves were catabolized during the winter lactation to provide energy for extra-mammary tissues, possibly as a result of depleted glucose precursor reserves. Non significant decline in total cholesterol during restriction in egypian native goats was found in Aboelmaaty et al., (2008) study. While statistically notable

higher concentrations (P<0.05) of the cholesterol have been found in the blood of the pregnant ewes comparing to the non-pregnant ones in ewes (Antunovic et al., 2004).Serum cholesterol concentrations in the spring were higher in cows receiving 6.55% dietary fat than in cows receiving 5.20% or 3.74% dietary fat, and tended to be greater on day (1) after calving in cows receiving 5.20% fat than in controls and were similar during the remainder of the treatment period (Lammoglia et al., 1996). Heifers fed the HF (Hereford Prepubertal) diet had greater cholesterol concentrations after 84 day of feeding and maintained the higher concentrations throughout the feeding period (Lammoglia et al., 2000). During the prepartum period the ewes fed propylene glycol showed lower plasma total cholesterol than ewes fed the control diet, while no differences were reported after lambing in the treatments (Chiofalo et al., 2005).

6.3.8 TRIGLYCERIDE.

There were significantly (P<0.05) differences between groups for triglyceride in both stages (start and at the end) of trial (I) when used glycerol as replacement, which found in 3rd group (glycerol) was increased at the start of experiment and decreased at the end of experiment (pregnancy period). Triglyceride concentration in 2nd and 4th groups at the end of experiment (pregnancy period) were increased, conversely than 1st and 3rd groups which decreased compared to both groups in the start of experiment respectively. Whereas in trial (II) when used glycerol as supplemental in diet, there were insignificantly (P<0.05) differences between groups in both stages (start and at the end) of experiment, which registered 3rd group (glycerol 10%) higher values in the both stages (start and at the end) of experiment respectively, while decreased in 2nd group (glycerol 20%) also at the two stages of experiment respectively. Whereas triglyceride concentrations were increased at the end of experiment (pregnancy period) groups compared to respective groups at the start of experiment. Our results in trial (I) agree with Piccione et al., (2009) study that found triglyceride level was decreased significantly during gestation in ewes. The significant decrease in serum triglycerides at the end of experiment (pregnancy period) could be explained as the effect of increased lipolysis which is hormonally regulated, and not an expression of energy deficiency (Holtenius and Hjort, 1990). While trial (II) results is in agreement with several authors (Mohamed and Abou-Zeina, 2008; Balikci et al., 2007;

Qoja and Šťastný, 2010) which observed similar result for triglyceride. This increase during pregnancy period may be due to insulin, which plays a direct role in adipose tissue metabolism during pregnancy and its responsiveness is reduced in ewes during late pregnancy, and the diminished responsiveness of the target tissue to insulin during late pregnancy predisposes the ewes to increase of triglyceride concentration (Schlumbohm et al., 1997). Mikula et al., (2008) mentioned to insignificant differences between groups were observed in plasma triglyceride concentration, and mean triglyceride concentration increased before calving and remained lower throughout the postpartal study period than during the dry period. Chiofalo et al., (2009) indicated the triglyceride concentration in propylene glycol group was lower than control in dairy goats, and this supporting our result for trial (I). While Ozdogan et al., (2006) found triglyceride value was higher in bulls group fed 2.5% tallow compared to bulls fed 2.5% cotton seed oil and without fat group. Cenesiz et al., (2006) observed no changes in concentrations of triglyceride were found among groups fed urea molasses mineral blocks in lambs. Mean of triglyceride serum concentrations were not significantly differences between groups in ewes take three levels of feed (Caldeira et al., 1999). Masek et al., (2007) mentioned the triglyceride levels significantly increased in the late milking period in dairy ewes. Chiofalo et al., (2005) observed that propylene glycol had no effect on plasma triglycerides during close-up dry period, and after lambing plasma triglycerides was decreased in all three treatments, an occurrence that could be attributed to the utilization of this metabolite in the uptake of the mammary gland (Ronchi et al., 1994). In dairy cows the changes in plasma triglycerides are a clearer parameter of a fatty liver, because triglyceride synthesis and storage is the principal metabolic fate of fatty acids when the hepatic capacity for oxidation is exceeded (Bertics et al., 1992).

6.3.9 SODIUM (Na).

Sodium is the main extra-cellular cation; most of it is present in the soft tissues, body fluids and haematic plasma. It participates in the maintenance the acid-base balance and in osmotic regulation. It plays a fundamental role in the absorption of sugars and amino-acids from the intestine and in the transmission of nerve impulses (Suttle, 1983).

Sodium concentration affected by glycerol insignificantly ($P<0.05$) that decreased in both stages (start and at the end) of trial (I) when used glycerol as replacement. And showed the Na concentrations at the end of experiment (pregnancy period) were decreased in all groups compared to respective groups at the beginning of experiment. Whereas in trial (II) when glycerol used as supplemental in diet, there were insignificant ($P<0.05$) differences between groups for sodium in both stages of experiment, which decreased in 2nd group (glycerol 20%) and increased in control group at the start of experiment. At the end of experiment (pregnancy period), Na was increased in 1st group (glycerol 30%) and decreased in 3rd group (glycerol 10%). Na concentrations at the end of experiment (pregnancy period) were increased in 1st and 2nd groups, while decreased in 3rd and 4th groups compared to respective groups at the beginning of experiment. Our result in trial (I) in agreement with, Ozdogan et al., (2006) that founded not statistically significant among the treatment groups for Na serum in fattening bulls fed tallow and cotton seed oil in diet and with Sobiech et al.,(2008) study that founded the level of Na fluctuated slightly but the observed difference were non significant and fell within the normal range in ewes nursing a single lambs or twins, and disagree with Milewski and Sobiech,(2009) study that registered a higher concentration of Na ($P<0.05$) in experiment group ewes fed dietary supplementation with saccharomyces cerevisiae dried yeast, while agree with our result for trial (II) especially in (glycerol 30% supplement) group. Sodium plays a vital role in maintaining osmotic pressure and acid-base balance as electrolytes (Tanritanir et al., 2009) which observed Na level did not changed before and after parturition in goats. While Mbassa and Poulsen, (1991) was found Na ion fluctuated only slightly during pregnancy and lactation. On the other hand, from Osman and Al-Busadah, (2003) study about differences in normal concentrations of serum biochemical parameters in camels, cows and ewes, observed the mean values of Na were significantly higher in ewes than cows. The differences in the concentrations of Na at the time of early and late pregnancy in a relationship to season were recorded in Marwari sheep (Mali et al., 1994). The evaluation of Na concentrations in comparison with day 1 post partum showed a statistically significant decrease ($P < 0.05$) with the lowest values on day 28 after parturition during puerperal period in goats (Krajnicakova et al., 2003). Bademkiran et al., (2008) mentioned in cows with self-sucking had significantly lower Na serum concentration. Antunovic et al.,

(2002) noted the concentrations of Na were statistical much lower in blood serum of pregnant and non-pregnant ewes comparing to the ewes in lactation. Elnageeb and Adelatif, (2010) observed serum Na during early flushing period compared to the initial values was increased significantly in both control and supplemented (received daily 500g of concentrate mixture crushed sorghum and cotton seed cake) groups, and at parturition in control group significantly increase was observed compared to the value measured during pregnancy, which agree with our result in trial (I), then the supplemented group had significantly higher serum Na level compared to the control, that agree with our result in trial (II). The Na increase in groups fed glycerol which noted in trial (II) was probably a consequence of blood concentration caused by fluid transfer from the vascular bed to the gastrointestinal tract (Russel and Chow, 1993). And the ruminal osmotic pressure increases over this period, and the rumen contents become hypertonic in relation to plasma, which results in the transfer of fluid along the concentration gradient. The return to hypotonic conditions in experimental ewes probably took a longer period, leading to haemoconcentration and a rise in Na concentrations (Milewski and Sobiech, 2009). The decrease in Na levels during pregnancy period that observed in our study could be related to increase in Na loss in urine due to the effect of progressive increase in progesterone level. Laidlaw et al., (1962) reported an increase in Na excretion during progesterone administration and they suggested that progesterone might antagonize aldosterone action in the kidney tubules. Also the decrease in Na during pregnancy may partly be related to the increase in fetal demands, an increase in accumulation of Na in the fetal lambs tissues during pregnancy (McDonald et al., 1979).

6.3.10 CHLORINE (CI).

Chlorine is essential in acid-base balance and osmotic regulation. Chlorine is fundamental in gastric secretion, where it is in the form of hydrochloric acid and as chloride salts, and associations with sodium and potassium (Suttle, 1983).

There were significant ($P<0.05$) differences between groups for Cl in both stages (at the start and the end) of trial (I) when used glycerol as replacement which registered lowest values in 3rd group (glycerol) for two stages of experiment. However Cl concentrations in 1st and 2nd groups were decreased at the end of experiment (pregnancy

period) compared to respective groups at the start of experiment, conversely than 3rd group which increased in pregnancy period. While in trial (II) when used glycerol as supplemental in diet, there were insignificant (P<0.05) differences between groups for Cl in both stages (at the start and end) of experiment that recorded highest value 2nd group (glycerol 20%) and lowest value was registered in control group at the start of experiment. Whereas at the end of experiment (pregnancy period) control group was increased then decreased in 2nd group. However Cl concentrations in 1st, 3rd and control groups were increased at the end of experiment (pregnancy period) compared to respective groups at the start of experiment, conversely than 2nd group which decreased in pregnancy period. The results of trial (II) agree with; Milewski and Sobiech, (2009) that recorded a higher Cl in ewes group fed saccharomyces cerevisiae dried yeast and Sobiech et al.,(2008) that mentioned the serum levels of Cl ions were similar in all ewes and did not changed over lactation. Bademkiran et al., (2008) mentioned in cows with self-sucking had significantly lower Cl serum concentration. On the other hand, from Osman and Al-Busadah, (2003) study about differences in normal concentrations of serum biochemical parameters in camels, cows and ewes, observed the mean values of Cl were significantly higher in ewes than cows. Hu and Murphy, (2004) suggest that the serum concentrations of chlorides are affected mostly by the supply of these ions in the diet. The levels of chloride ions are closely correlated with the parameters of acid-base balance (Sobiech et al., 2008), and the small fluctuations in this parameter observed in the present study, which decrease little throughout pregnancy in first trial. The various increases in Cl levels that noted in ewes fed supplemental glycerol in trial (II) was probably a consequence of blood concentration caused by fluid transfer from the vascular bed to the gastrointestinal tract (Russel and Chow, 1993). And the ruminal osmotic pressure increases over this period, and the rumen contents become hypertonic in relation to plasma, which results in the transfer of fluid along the concentration gradient. The return to hypotonic conditions in experimental ewes probably took a longer period, leading to haemoconcentration and a rise in and Cl concentrations (Milewski and Sobiech, 2009).

6.3.11 POTASSIUM (K).

Potassium is fundamental (along with sodium, chlorine) in the osmotic regulation of body fluids and in the acid-base balance in the organism. It is the main intracellular cation and plays a role of primary importance in nerve and muscle excitability (Suttle, 1983).

There were significant ($P<0.05$) differences between groups for K in two stages (at the start and the end) of trial (I) when used glycerol as replacement which found in 4^{th} group a higher value than other groups in the start and the end of experiment. While K concentrations at the end of experiment (pregnancy period) were increased in 1^{st} group and decreased in 2^{nd} , 3^{rd} and 4^{th} groups compared to respective groups at the start of experiment. While in trial (II) when used glycerol as supplemental in diet, there were significant ($P<0.05$) differences between groups for K at the start of experiment that recorded 2^{nd} group (glycerol 20%) a higher value and lower value was recorded in control group. Whereas at the end of experiment (pregnancy period) were observed insignificant ($P<0.05$) differences between groups for K which increased in 2^{nd} group and decreased also in control group. While K concentrations at the end of experiment (pregnancy period) were decreased in all experimental groups compared to respective groups at the start of experiment. Effect of glycerol on Potassium (K) had been not clear in trial (I). Some authors were reported by (Kulcu and Yur, 2003) study there were significant differences between open and pregnancy periods for serum K concentration in sheep, and (Mbassa and Poulsen, 1991) observed K was fluctuated only slightly during pregnancy and lactation. It was considered that there was no obvious effect resulting from feeding because these values were within the normal range (Altintas and Fidanci, 1993). Sobiech et al., (2008) observed the level of K was fluctuated slightly but the observed difference were non significant and fell within the normal range in ewes nursing a single lambs or twins. From Osman and Al-Busadah, (2003) study about differences in normal concentrations of serum biochemical parameters in camels, cows and ewes, serum potassium concentration was significantly higher in ewes when compared with she-camels and cows. Bademkiran et al., (2008) mentioned in cows with self-sucking had significantly lower K serum concentration. Elnageeb and Adelatif, (2010) observed serum K level was significantly lower during early flushing compared to the initial values in both control and supplemented (received daily 500g of concentrate mixture crushed sorghum and cotton seed cake) group of ewes, also in

both groups there were a gradual significant increase in K level after mating and during early and mid gestation and then it decreased significantly during late gestation. Tanritanir et al., (2009) noted K level did not change after parturition in goats. Ozdogan et al., (2006) found differences for K levels among the treatment groups were not statistically significant when used cotton seed and tallow in fattening bulls. Antunovic et al., (2004) indicated to statistically significant higher concentrations of K have been marked in the lactation and non-pregnant ewes comparing to the pregnant ones, which agree with our results during pregnancy period in trial (II). The depression of K concentrations in groups during pregnancy period could be attributed to pregnant ewes was to be related to metabolism disorders possibly occurring at the end of pregnancy - which in turn may lead to diverse pathologic deviations of metabolites in the blood (Antunovic et al., 2002), or might be increased aldosterone level caused by low levels of Na and Cl (Lippmann, 1995). While K concentrations rise in groups fed glycerol maybe attributed to hormonal changes associated with increase in Na (that observed in trial, II) levels. It has been reported by (Michella et al., (1988) that plasma aldosterone level remained low in ewes maintained on high sodium.

6.3.12 CALCIUM (Ca).

Calcium is the most abundant mineral element in the animal body and it is fundamental for the activity of many enzyme systems, coagulation of blood, transmission of nerve impulses, contraction of muscles, flocculation of casein in the stomach and many others (NRC, 1985). The absorption of calcium in the intestine increases with the requirements of the animals and when the content of the element decreases in the diet (Braithwaite, 1982).

There were significant ($P<0.05$) differences between groups for Ca in both stages (at the start and at the end) of trial (I) when used glycerol as replacement which recorded highest values in 4th group in two stages of experiment. Ca concentrations in all groups were decreased at the end of experiment (pregnancy period) comparison with respective groups at the start of experiment. Whereas in trial (II) when used glycerol as supplemental in diet, the insignificant ($P<0.05$) differences between groups noted in both stages (start and the end) of experiment, that recorded highest values in 2nd group (glycerol 20%) and lowest value was recorded in 3rd group (glycerol 10%) at the start of experiment. At the end of

experiment (pregnancy period), Ca concentration was decreased 1st group (glycerol 30%) then increased in control group. Ca concentrations were decreased in 1st and 2nd groups, conversely than 3rd and control groups were increased at the end of experiment (pregnancy period) compared to respective groups at the start of experiment. Our result in trial (I) disagree with Moghaddam and Hassanpour, (2008) that found the level of Ca concentration in prepartum period was higher than postpartum period significantly ($P<0.01$) in ewes. Sabra and Hassan, (2008) observed that flushing of Egyptian Barki ewes with 200g/ewe daily of a balance mixture composed of 58% crushed yellow corn and 41% crushed soybean was improved the blood concentration of calcium. The concentration of calcium varied significantly during the milking period with the highest value in the late and the lowest in the mid milking period, however they were always within the reference values in dairy ewes (Masek et al., 2007). Several authors reported various results about Ca levels during pregnancy and lactation. Some of them were found that Ca levels decreased (Ahmed et al., 2000; Liesegang et al., 2007; Mbassa and Poulson, 1991). On the other hand Kadzere et al., (1997) reported that Ca concentration in plasma increased as gestation progressed and decreased after kidding. While in Tanritanir et al., (2009) study did not found statistical differences between before and after parturition at Ca levels in goats. Also Ozdogan et al., (2006) did not observe statistical differences among treatment groups when fed cottonseed and tallow in fattening bulls. Antunovic et al., (2004) was found higher concentrations of Ca in the blood of non-pregnant ewes, which agree with our results in trial (I). The decrease of Ca levels during pregnancy period in trial (I) results can be related to risks associated with parturition hypocalcemia in ruminants (Kaneko, 1997). Yano et al., (1991) have established that over the pregnancy the needs for Ca are increased in parallel with increase of the Ca absorption in the intestines. Elnageeb and Adelatif (2010) observed serum Ca were significantly lower in both groups for early flashing period and after mating, and Ca level increased significantly in parturition in both groups of ewes, then supplemented group maintained significantly higher serum Ca levels compared to control group. The reason of Ca increase in ewes group fed glycerol or in pregnancy period can be attributed to the fact that despite plentiful dietary intake of Ca, the ewes were unable to absorb sufficient amount of Ca during gestation. It has been pointed out (Braithwaite, 1983) that the rate of absorption of Ca from the intestine increase in early pregnancy and decrease with the

advance of pregnancy and they added that endogenous loss of Ca increase with the advance of pregnancy. Or could be related to increase in Ca mobilization from the skeleton to meet the higher demand of Ca (Braithwaite, 1983). Moreover, estrogen increase Ca retention and in the process (Swenson and Reece, 1970).

6.3.13 PHOSPHOR (P).

This mineral is of vital importance in the energy metabolism, in fact, it participates in the formation of sugar-phosphates and adenosine diand tri- phosphates. It is a component of phosphoproteins, nucleic acids and phospholipids. The content of phosphors in the animal body is lower than that of calcium (Suttle, 1983). Phosphor is a component of phospholipids, which are important in lipid transport and skeleton and dent formation (Krajnicakova et al., 2003).

The results of trial (I) when used glycerol as replacement, significantly ($P<0.05$) differences between groups were observed for Phosphor at the start of experiment that recorded 4th group a higher value Glycerol not effected on Phosphor concentration at the end of experiment that indicate insignificant ($P<0.05$) differences between groups, while 4th group also registered a higher value also. Phosphor concentrations at the end of experiment (pregnancy period) were increased in 1st group and decreased in all other groups (2nd, 3rd, and 4th) than respective groups at the start of experiment. While in trial (II) when used glycerol as supplemental in diet, there were insignificant ($P<0.05$) differences between groups were observed for Phosphor in two stages (start and at the end) of experiment that recorded 2nd group (glycerol 20%) a higher value and lower value was recorded in control group at the start of experiment. At the end of experiment (pregnancy period), P concentration was higher in 2nd group and lower in 3rd group (glycerol 10%). Phosphor concentrations at the end of experiment (pregnancy period) were decreased in all groups (except 4th group which increased) compared to respective groups at the start of experiment. Several researchers reported that no significant differences were observed at the phosphor levels in the different stages of growth, reproduction, pregnancy and lactation (Kaushik and Bugalia, 1999; Krajnicakova et al., 2003), other researchers informed that phosphor level during late gestation and postpartum significantly increased in ewes and goats (Ahmed et al., 2000; Mbassa and Poulsen, 1991). Other authors reported that yeast

supplements had no effect on the levels of the Phosphor (Galip, 2006; Payandeh and Kafilzadeh, 2007; Milewski and Sobiech, 2009). Tanritanir et al., (2009) was found P concentrations increased after parturition. While Ozdogan et al., (2006) did not observe statistical differences among treatment groups when fed cottonseed and tallow in fattening bulls. Bademkiran et al., (2008) mentioned to cows with self-sucking had significantly lower serum concentration of Phosphor. Masek et al., (2007) observed Phosphors values were significantly higher in early milking period with respective to the mid or late milking period in dairy ewes. Elnageeb and Adelatif (2010) noted in desert ewes, the serum P level in supplemented group (that received daily 500g of concentrate mixture of crushed sorghum and cotton seed cake) revealed significantly higher value at the end of flushing period, and there were a significant decrease in P levels during late gestation, also supplemented ewes showed significantly higher level compared to the control during early gestation. The rise levels of P concentrations in groups fed glycerol with various levels could be attributed to an increase in the rate of mobilization of P in maternal circulation (Braithwaite et al., 1983), or maybe the concentration of plasma P and the rate of salivary secretion were responsible for endogenous faecal excretion P and regulation of P balance (Ternouth, 1989). Whereas depression phosphor levels during pregnancy period is reasonable as Braithwaite et al., (1983) reported that during pregnancy in sheep, absorption and demands of P increased and the endogenous loss of P in urine and faeces increased. Many factors associated with diet composition can affect minerals utilization and bioavailability.

6.3.14 MAGNESIUM (Mg).

Magnesium is involved in many biochemical processes, including activation of phosphates and participation in carbohydrate metabolism, it activates enzymes necessary for the metabolism of carbohydrates and amino acids, plays an important role in neuromuscular contractions, helps regulate the acid-alkaline balance in the body, aids during bone growth and is necessary for proper functioning of the nerves and muscles, including those of the heart. Sufficient amounts of magnesium are needed in the conversion of blood sugar into energy (Klasing, 1998).

There were significant (P<0.05) differences between groups for Mg in both stages (at the start and the end) of trial (I) when used glycerol as replacement that recorded lowest value in 3rd (glycerol) group for two stages of experiment. However Mg concentrations at the end of experiment (pregnancy period) were decreased in 3rd group (glycerol) and increased in 1st, 2nd and 4th groups when comparing with respective groups at the start of experiment. Whereas in trial (II) when used glycerol as supplemental in diet, the results indicate significantly (P<0.05) differences between groups for Mg at the start of experiment that recorded lowest value in 1st group (glycerol 30%) and highest value was recorded in control group. However Mg concentrations at the end of experiment (pregnancy period) was decreased in 2nd group (glycerol 20%) and increased in control group insignificantly (P<0.05). Mg concentrations in 2nd, 3rd and control groups were lower, conversely than 1st group (it was higher) at the end of experiment (pregnancy period) compared to respective groups at the start of experiment. Results indicate that Mg affected by glycerol which decrease in most group when used glycerol with various levels. Mg levels decreased very little during pregnancy period in this study, but this decreased was considered to be statistically significant. (Kulcu and Yur, 2003) mentioned to significant differences between pregnancy and the lactation period for serum Mg concentration in sheep. Several authors reported that the levels of Mg increased sometimes (Ahmed et al., 2000; Kadzere et al., 1997) and decreased in other times in relation to different periods of pregnancy and lactation (Mbassa and Poulsen, 1991). Other authors reported that yeast supplements had no effect on the levels of the Mg (Galip, 2006; Payandeh and Kafilzadeh, 2007; Milewski and Sobiech, 2009). Ozdogan et al., (2006) did not observe statistical differences among treatment groups when fed cottonseed and tallow in fattening bulls. Masek et al., (2007) noted the values of Mg were constant during the milking period. Brito et al., (2006) was observed higher Mg levels in late lactation. Elnageeb and Adelatif (2010) observed the serum Mg level was a significant decrease during early flashing in supplemented group (which received daily 500g of concentrate mixture of crushed sorghum and cotton seed cake), also after mating was founded a significant decrease in Mg level, and indicated the supplemented group had significantly higher serum Mg levels compared to the control group during and mid gestation. In our study results, depression Mg concentrations in animal groups fed various level of glycerol can be explained to Mg level is influenced by

the level of protein in diet (Hendricks et al., 1970). Or may not be explained in terms of an imbalance between supply and demand. However, factors influencing the absorption of Mg from the gut, which include dietary glycerol, could be implicated. While decrease Mg levels in most groups during pregnancy in our study may not be explained by the reciprocal relation with serum Ca level, as the levels of both minerals decreased during pregnancy period. Therefore, the decrease in serum Mg in presumably related to haemodilution which usually occurs during pregnancy (Elnageeb and Adelatif, 2010).

6.4 GYCEROL EFFECT ON HAEMATOLOGY PARAMETERS.

The hematological tests served as information base for animal health assistance. It has been reported that regardless of age, sex and climate, ruminants reared under traditional husbandry system have low hematological values compared to those reared under modern husbandry (Coles, 1980). Low nutritional grassland pasture, stress, parturition and climatic factors greatly alter the blood values of ruminants (Radostits et al., 1994).

6.4.1 WHITE BLOOD CELLS (WBC).

There were insignificant ($P<0.05$) differences between groups for WBC in two stages (at the start and estrus phase) of trial (I) when used glycerol as replacement, which recorded lower value in 3rd group at the start of experiment, while in estrus phase lower value was registered in 1st group, whereas at the end of experiment (pregnancy period) significant ($P<0.05$) differences between groups were founded that recorded a lower value in 2nd group. Generally WBC groups in estrus phase were highest than respective groups in the start of experiment, then all groups were decreased at the end of experiment (pregnancy period) respectively. While in trial (II) when used glycerol as supplemental in diet, there were insignificant ($P<0.05$) differences between groups in both stages (at the start and the end) of experiment, which 1st group (glycerol 30%) was registered lower value at the start of experiment, and higher value was recorded at the end of experiment (pregnancy period). Generally WBC groups (except 1st group) at the end of experiment (pregnancy period) were depressed than respective groups at the start of experiment. Milewski and Sobiech (2009) were found WBC count was higher during lactation in ewes fed dietary supplementation with saccharomyces cereviae dried yeast. Bhatti et al., (2009) was observed significantly

differences in WBC between treatments in Nili-ravi buffalo heifers fed on mott grass and berseem fodder substituted with saltbush (Atriplex Amnicola). While Faixova et al., (2007) noted that WBC was increased in lambs given basic diet. Soch et al., (2010) observed the higher counts of WBC were contained in the spring season in sheep. Al-Mujalli (2008) mentioned to WBC was decreased in sheep associated with low dose feeding of Anagallis Arvensis. The comparative hematological parameters from pregnant and non-pregnant red and fallow deer are showed Poljicak-Milas et al., (2009) study that total leukocyte count (WBC) was the highest in non-pregnant red deer hinds, while in pregnant hinds reduced WBC count was determined. Fallow deer does have lower WBC than red deer hinds, with the lowest WBC value in non-pregnant fallow deer females. Pisek et al., (2008) indicated decrease in count of WBC was registered during pregnancy in sheep which agreed with our results in pregnancy period for two trials. The reason of decrease WBC during pregnancy can be attributed to both lymphocytes and granulocytes participated in a decrease the count of WBC during pregnancy. And a decrease in the values of WBC count was the most marked, this finding corresponds to the value measured in pregnant rhesus monkeys (Macaca mulatta) compared to non-pregnant animals (Rogers et al., 2005).

6.4.2 LYMPHOCYTE (LY).

There were insignificant ($P<0.05$) differences between groups for lymphocyte in both stages (at the start and estrus phase) of trial (I) when used glycerol as replacement, which increased in 3rd group (glycerol) at the start of experiment, while in estrus phase, 2nd group was higher than others, at the end of experiment (pregnancy period) LY affected by glycerol which decreased significantly ($P<0.05$) in 3rd group (glycerol). Results showed LY respective groups were decreased in estrus phase when compared to starting of experiment groups, then increased at the end of experiment (pregnancy period) respectively. While in trial (II) when used glycerol as supplemental in diet, results indicate there were insignificant ($P<0.05$) differences between groups at the start of experiment which registered 3rd group (glycerol 10%) a higher value while in control group was depressed. Whereas at the end of experiment (pregnancy period), the differences between groups were significantly ($P<0.05$) which recorded a higher value in control group and lower value was founded in 1st group (glycerol 30%). LY results showed 1st, 2nd and 3rd groups were lower

conversely than control group which increased at the end of experiment when compared with respective groups at the start of experiment. Pisek et al., (2008) observed the effect of selenium supplementation in sheep was changed in percentage of lymphocytes in the period of non-pregnancy and during pregnancy which increased during pregnancy that's agree with our results in trial (I). During comparative study for hematological values in pregnant and non-pregnant red, Cervus elaphus, and fallow deer Dama dama females, Polljicak-Milas et al., (2009) mentioned pregnant hinds had lower lymphocyte count than non-pregnant ones, while pregnant does had more than twice lymphocyte count in their blood than non-pregnant animals. Masek et al., (2009) was recorded higher lymphocyte percentage for European mouflon than domestic sheep in Croatia. Peripartal changes in intraepithelial lymphocytes in ewes were studied in the uterine tissue by Nasar et al., (2002), who determined a decrease in the white blood cell count in the uterine stroma. Lipecka et al., (2010) showed that offspring born to seropositive mothers were higher lymphocyte count than lambs born to seronegative ewes, and in older lambs lymphocyte count was significantly lower in MVV+ group (maedi-visna virus seroposetive) than in the MVV- group (maedi-visna virus seronegative) when studied some haematological parameters in blood of lambs born to maedi-visan virus-infected and uninfected ewes. The high lymphocyte counts in the animal groups fed glycerol with various levels might be attributed to stress and immune response to the nutrition. And during pregnancy the process of the lymphocyte recirculation is a critical element in the integration of systematic immune responses (Mackay, 1993).

6.4.3 GRANULOCYTE (GR).

For trial (I) results when used glycerol as replacement, insignificant ($P<0.05$) differences between groups were founded for GR at the start of experiment which decreased in glycerol group, but glycerol was increased insignificantly ($P<0.05$) in the estrus phase, while GR affected by glycerol at the end of experiment (pregnancy period) which increased significantly ($P<0.05$). Generally all GR groups were increased in the estrus phase then decreased at the end of experiment (pregnancy period) respectively when compared with groups at the start of experiment. Whereas in trial (II) when used glycerol as supplemental in diet, insignificant ($P<0.05$) differences between groups were founded for

GR at the start of experiment which decreased in 3rd group (glycerol 10%) and increased in control group, while significantly (P<0.05) differences between groups were observed at the end of experiment (pregnancy period) which increased in 1st group (glycerol 30%) and decreased in control group. Generally all GR groups were increased (except control group which decreased) at the end of experiment compared to respective groups at the start of experiment. Lipecka et al., (2010) showed that offspring born to seropositive mothers were lower granulocyte count than lambs born to seronegative ewes, and in older lambs granulocyte count was significantly lower in MVV+ group (maedi-visna virus seroposetive) than in the MVV- group (maedi-visna virus seronegative) when studied some haematological parameters in blood of lambs born to maedi-visan virus-infected and uninfected ewes.

6.4.4 RED BLOOD CELLS (RBC).

There were insignificant (P<0.05) differences between groups for RBC in three stages (start, estrus and at the end) of trial (I) when used glycerol as replacement. RBC affected by glycerol which increased in the start of experiment and estrus phase respectively, but glycerol not effected on RBC at the end of experiment (pregnancy period), while all RBC groups were decreased in estrus phase then increased at the end of experiment (pregnancy period) respectively comparison with start of experiment groups. While in trial (II) when used glycerol as supplemental in diet, there were insignificant (P<0.05) differences between groups for RBC in both stages (start and at the end) of experiment. RBC affected by glycerol 30% which increased in the start of experiment and at the end of experiment (pregnancy period), whereas 3rd group was depressed at the start of experiment and 2nd group was lower at the end of experiment. All RBC groups at the end of experiment (pregnancy period) were decreased compared to respective groups at the start of experiment. Milewski and Sobiech (2009) was found RBC count was higher during lactation in ewes fed dietary supplementation with saccharomyces cereviae dried yeast, which is agreed with our results in trial(I). While Lipecka et al., (2010) showed that in young lambs RBC was significantly higher in MVV+ group (maedi-visna virus seroposetive) than in the MVV- group (maedi-visna virus seronegative) when studied some haematological parameters in blood of lambs born to maedi-visan virus-infected and

uninfected ewes. Bhatti et al., (2009) was observed significantly differences in RBC between treatments in Nili-ravi buffalo heifers fed on mott grass and berseem fodder substituted with saltbush (Atriplex Amnicola), which found RBC count in heifers was higher on (Mott) group and (Mott+saltbush) group diets. Significantly ($P<0.05$) lower RBC count was observed on (Berseem+saltbush) group and (Berseem alone) group. Al-Mujalli (2008) mentioned RBC was decreased in sheep associated with low dose feeding of Anagallis Arvensis. Faixova et al., (2007) noted that RBC was decreased in lambs given basic diet. In bighorn sheep, young animals had a significantly higher RBC mass than adult animals, and then adult females like young animals had a significantly higher RBC mass than male animals (Borjesson et al., 2000). While Masek et al., (2009) was registered higher RBC count for European mouflon than domestic sheep in Croatia. Whereas Binev et al., (2007) indicated that red blood cells count was highest in the lambs of Ile de France breed. The comparative hematological parameters from pregnant and non-pregnant red and fallow deer are showed Poljicak-Milas et al., (2009) study that pregnant females of both species had lower RBC count, which agreed with our results during pregnancy for trial (II). The decrease of RBC during pregnancy period can be explain to Cross et al., (1988) stated that spleen is contracted due to adrenaline release when manual restraint or a crush is used. Which the fear and excitement, consequently higher adrenalin release and spleen contraction, could have an impact in pregnancy period on total RBC, or during the pregnancy some metabolic changes occur that may alter physiological range of blood constituents (Jainudeen and Hafez,1994; Ei-Sherif and Assad,2001). In present study, glycerol using as replacement energy and as supplemental in diet caused an increase in the count of RBC in glycerol group for two first stages in trial (I), and for glycerol (30%) group in both stages of trial (II). Similar results were reported by Dobicki et al., (2005) and Heinrichs et al., (2003) who examined the effect of supplements on the health status of cattle.

6.4.5 HEMOGLOBIN (HGB).

Insignificant ($P<0.05$) differences between groups were founded for HGB in the three stages (start, estrus and at the end) of trial (I) when glycerol used as replacement. HGB affected by glycerol which increased at the start of experiment and estrus phase,

whereas not effected at the end of experiment (pregnancy period). Generally three HGB groups (1^{st}, 2^{nd} and 4^{th}) were decreased in estrus phase then increased at the end of experiment (pregnancy period) respectively compared to start of experiment groups, conversely than 3^{rd} (glycerol) group which increased in estrus phase then decreased at the end of experiment comparison with start of experiment group. While in trial (II) when glycerol used as supplemental in diet, also insignificant ($P<0.05$) differences between groups were founded for HGB in both stages (start and at the end) of experiment. HGB concentration was higher in 2^{nd} group (glycerol 20%) and lower value was recorded in 3^{rd} group (glycerol 10%) at the start of experiment. Whereas at the end of experiment (pregnancy period) 3^{rd} group (glycerol 10%) was higher and in 2^{nd} group was decreased. Two HGB groups (1^{st} and 2^{nd}) were decreased and two others (3^{rd} and control) were increased at the end of experiment (pregnancy period) compared with respective groups at start of experiment. Milewski and Sobiech (2009) were found HGB count was higher during lactation in ewes fed dietary supplementation with saccharomyces cereviae dried yeast. El-sherif and Assad (2001) indicated to haemoglobin concentration was highest during pregnancy, which is agreed with our results in trial (I). Bhatti et al., (2009) was observed significantly differences in HGB between treatments in Nili-ravi buffalo heifers fed on mott grass and berseem fodder substituted with saltbush (Atriplex Amnicola), which found HGB contents in heifers were higher on (Mott+ Berseem+ Saltbush) group followed by(Berseem alone) group, (Mott+saltbush) group, (Berseem+saltbush) group and (Mott) group. Al-Mujalli (2008) mentioned HGB was decreased in sheep associated with low dose feeding of Anagallis Arvensis. Young bighorn sheep had a significantly higher HGB mass than adult animals, and then adult females like young animals had a significantly higher HGB mass than male animals (Borjesson et al., 2000). While Masek et al., (2009) was registered higher HGB for European mouflon than domestic sheep in Croatia. Whereas Binev et al., (2007) indicated that haemoglobin was highest in the lambs of Mouton Charollais breed. Soch et al., (2010) observed the significant differences between systems for haemoglobin which found higher value in ecologic system in sheep. The means of haemoglobin values observed in the four groups of West African dwarf sheep used in Durotoye and Oyewale, (2000) study which showed the highest was in the pregnant, than in the lactation ewes, the dry ewes and the rams. While Obidiki et al., (2009) observed no

significant changes were recorded in haemoglobin concentration between day 5 and 30 postpartum in West African dwarf ewes. The comparative hematological parameters from pregnant and non-pregnant red and fallow deer are showed Poljicak-Milas et al., (2009) study that pregnant females of both species had lower HGB concentration than non-pregnant females, which agreed with our results during pregnancy for trial (II). Abdelatif et al.,(2009) observed the haemoglobin concentration increased significantly with the advance of pregnancy in both groups of ewes, and the supplemented ewes(that's received daily 500g concentrate mixture of crush sorghum grain and cottonseed cake) maintained higher value of haemoglobin during pregnancy and lactation. The observation increase in HGB concentration in pregnancy period in present study (trial I) confirms the finding in Merino ewes (Jelinek et al., 1986) and supports the observation in Barki ewes (El-Sherif and Assad, 2001). The rise of haemoglobin concentrations during pregnancy in trial (I) may be attributed to the availability of certain nutrients needed for formation of RBC, this may be most demanding in the last trimester of pregnancy for supply of nutrients and blood to the fetus and may increase many fold to meet the requirements of the dam under nutritional stress (Khan et al., 2002). The higher HGB values were found in the pregnant ewes indicates that contrary to the common situation in humans, pregnancy does not exert any negative influence on the HGB value in the sheep. Reynold (1953) have reported the development of hydremia and anaemia in women during pregnancy. A similar report of non-development of anaemia in local breeds of cattle and goat has earlier been made (Reynold, 1953; Durotoye, 1987). While decrease HGB levels during pregnancy in two glycerol groups of our results for trial (II) could be attributed to the haemodilution effect resulting from an increase in plasma volume (Mbassa and Poulsen, 1991).

6.4.6 HEMATOCRITE (HCT).

Differences between groups for HCT were insignificantly ($P<0.05$) in all stages (start, estrus and at the end) of trial (I) when used glycerol as replacement. Glycerol effected on HCT which increased in two firs stages (at the start and estrus phase) of experiment, but not effected at the end of experiment (pregnancy period). The comparison with three stages of experiment we observed, all trial groups in estrus phase were lower than at the start of experiment groups respectively, after then they were increased at the end

of experiment (pregnancy period). Whereas in trial (II) when used glycerol as supplemental in diet, there were significant ($P<0.05$) differences between groups for HCT at the start of experiment which increased in 2nd group (glycerol 20%) and decreased in 3rd group (glycerol 10%). While at the end of experiment (pregnancy period), the differences between groups were insignificantly ($P<0.05$) that increased in control group and decreased in 1st group (glycerol 30%). HCT levels in all trial groups at the end of experiment (pregnancy period) were lower than respective groups at the start of experiment. Bhatti et al., (2009) observed significant differences in HCT between treatments that recorded higher value in animals group fed (Mott+Berseem+saltbush). Lipecka et al., (2010) showed that in young lambs HCT was significantly higher in MVV+ group (maedi-visna virus seroposetive) than in the MVV- group (maedi-visna virus seronegative) when studied some haematological parameters in blood of lambs born to maedi-visan virus-infected and uninfected ewes. While Al-Mujalli, (2008) mentioned HCT was decreased in sheep associated with low dose feeding of Anagallis Arvensis. Young bighorn sheep had a significantly higher HCT mass than adult animals, and then adult females like young animals had a significantly higher HCT mass than male animals (Borjesson et al., 2000). Masek et al., (2009) was registered higher HCT count for European mouflon than domestic sheep in Croatia. While Binev et al., (2007) indicated that haematocrit was highest in the lambs of Ile de France breed. Obidiki et al., (2009) observed no significant changes were recorded in haematocrit levels between day 5 and 30 postpartum in West African dwarf ewes. El-Sherif and Assad, (2001) indicated that during pregnancy HCT was started to increase significantly, and from 10th week to parturition also increased in pregnant ewes. The means of haematocrit levels observed in the four groups of West African dwarf sheep used in Durotoye and Oyewale, (2000) study which showed insignificantly higher mean PCV was recorded for the lactating ewes than for the dry ones, and significantly ($P<0.05$) higher mean PCV value was observed for the pregnant ewes relative to either the dry or the lactating ones, that's mean pregnant ewes group was registered the highest percentage of PCV. The comparative hematological parameters from pregnant and non-pregnant red and fallow deer are showed Poljicak-Milas et al., (2009) study that pregnant females of both species had lower HCT value than non-pregnant females, which agreed with our results during pregnancy for trial (II). Abdelatif et al.,(2009) indicated to significantly decrease in HCT during early flashing

and early and mid-gestation in both ewes group (control and supplemented), then in supplemented ewes group (that's received daily 500g concentrate mixture of crush sorghum grain and cottonseed cake) HCT was significantly higher at gestation, parturition and lactation phase as compared to respective control group values. Milewski and Sobiech, (2009) was found haematocrit value was higher during lactation in ewes fed dietary supplementation with saccharomyces cereviae dried yeast. The rise of haematocrit values during pregnancy in trial (I) could be attributed to the availability of certain nutrients needed for formation of RBC, this may be most demanding in the last trimester of pregnancy for supply of nutrients and blood to the fetus and may increase many fold to meet the requirements of the dam under nutritional stress (Khan et al., 2002). While the decrease in HCT values during pregnancy in groups of our results for trial (II) could be attributed to the haemodilution and is supported by Robinson et al., (1978). The observed decrease in HCT also agree with the results reported for gestation Barki ewes (Hassan et al., (1987) and the finding for Probstheida breed pigmy goat (Fortagne and Schafer, 1989). On the other hand increase HCT in ewes group fed glycerol with various levels can be explain to the need for excess RBC to transport oxygen to meet the enhanced metabolism (Abdelatif et al., 2009).

7. CONCLUSIONS:

From the results obtained we can conclude the following:

1) Feeding glycerol 20% as energy replacement in diet during breeding season was effected relatively on estrus length which decreased significantly, while gravidity percentage, pregnancy length, number of lambs, twins lambing, male lambs, fertility, prolificacy and fecundity were increased insignificantly, and single lambing was decreased insignificantly, whereas female lambs and female lambs weight were affected by glycerol relatively that increased significantly, while glycerol increase total lambs weight significantly. Glycerol 30% supply in diet decrease estrus length significantly, while glycerol supplementation with three levels increased gravidity insignificantly, in pregnancy length glycerol 10% was higher insignificantly than others, whereas glycerol 30% increase number of lambs significantly, different glycerol levels (10%, 20% and 30%) increase insignificantly single, twins and triplet lambing respectively, glycerol 10% increase male lambs and male lambs weight insignificantly, while glycerol 20% increase female lambs insignificantly, whereas glycerol 30% increase female lambs weight and total lambs weight significantly, and glycerol with three levels were increase fertility insignificantly, while glycerol 30% increase prolificacy and fecundity insignificantly.
2) Glycerol 20% using as energy replacement was associated with positive and negative effect on hormones profile which decreased FSH insignificantly at the start and at the end (pregnancy period) of trial, while decreased LH in estrus phase insignificantly, whereas progesterone was higher in two stages (at the start and estrus phase) significantly while in pregnancy period was higher insignificantly, glycerol increase estradiol at the start and estrus phase then decrease in pregnancy period insignificantly, however glycerol effect on prolactin was not clear. Using glycerol 30% as energy supplement increase FSH and progesterone concentrations in both stages of trial (at the start and pregnancy period) insignificantly, while glycerol 20% decrease insignificantly estradiol concentrations in two stages of experiment, glycerol 10% decrease LH concentrations at the start of experiment while glycerol 30% decrease LH in pregnancy period insignificantly, glycerol 30%

effect was insignificantly on prolactin hormone which decrease at the start of experiment and increase in pregnancy period.

3) In respect of biochemical properties of blood, glycerol 20% replacement did not effected ALP and total protein clearly although found significant differences between groups, while glycerol effected ALT and AST significantly which decreased in pregnancy period, whereas glycerol effected urea and glucose insignificantly which increased at the start of trial and decreased in pregnancy period, cholesterol affected by glycerol insignificantly that decreased in two stages of trial, while triglyceride affected by glycerol significantly which increased at the start of trial and decreased in pregnancy period. Glycerol 30% supply in diet effect on enzymes profile was higher significantly in ALP and higher insignificantly effect on ALT and AST in both stages of trial, while glycerol 20% increase total protein in two stages of experiment, as regard to urea and glucose, glycerol 20% decrease urea significantly and glucose insignificantly at the start of trial, whereas glycerol 30% decrease urea significantly and glucose insignificantly in pregnancy period, while cholesterol was insignificantly higher in glycerol 10% treatment at the start of trial and in glycerol 30% treatment in pregnancy period, then glycerol 10% increase triglyceride insignificantly in both stages of experiment.
4) For minerals profile, glycerol 20% replacement decline Na concentration in two stages of trial insignificantly, while glycerol effected on Cl and Mg concentrations significantly which decreased in two stages of trial, glycerol effect on K, Ca and P concentrations was not clear although found significant differences between groups. Na affected by glycerol supplementation treatments insignificantly which decreased in glycerol 20% treatment at the start of trial and increased in glycerol 30% treatment in pregnancy period, while glycerol effected insignificantly on Cl in both stages of experiment which increased at the start of trial and decreased in pregnancy period, whereas glycerol 20% increase K significantly at the start of trial and insignificantly in pregnancy period, Ca concentrations were higher in glycerol 20% treatment at the start of trial and lower in glycerol 30% treatment in pregnancy period insignificantly, glycerol 20% increase P insignificantly in both stages of experiment, and Mg was lower significantly in glycerol 30% treatment at the start

of trial then decreased in glycerol 20% treatment in pregnancy period insignificantly.

5) In reference to haematology profile, WBC affected by glycerol 20% replacement insignificantly that decreased at the start of trial, while glycerol increase LY at the start of trial insignificantly and at pregnancy period was decreased significantly, conversely than glycerol effect on GR which decrease at the start of trial insignificantly and increase in pregnancy period significantly, whereas glycerol increase RBC, HGB and HCT in two stages (at the start and estrus phase) of trial insignificantly. WBC affected by glycerol 30% supply in diet insignificantly which decreased at the start of trial and increased in pregnancy period, while glycerol 20% effected insignificantly at the start of trial which increased LY and decreased GR, whereas glycerol 30% effected significantly in pregnancy period which decreased LY and increased GR, RBC affected by glycerol 30% insignificantly which increase in both stages of experiment, while glycerol 20% increase both of HGB and HCT insignificantly at the start of trial, whereas glycerol 10% increase both of HGB and HCT in pregnancy period insignificantly.
6) The data imply that glycerol 20% should be delivered as a replacement and addition (30%) glycerol to the diet in ewes fed as a component of transition ewes diets especially during breeding season in two trials of study to improvement reproduction characteristics and related hormones with biochemical and mineral properties addition to haematological profile which are important for metabolism process during this period (breeding season).
7) Improving the energy status of ewes during the breeding season by adding a safe dietary supplement such as glycerol may have a positive effect on reproductive function.

8. RECOMMEDATIONS:

Upon the information obtained from the literature and this research data the following recommendations could be considered:

1) The number of animals utilized per treatment in this study might not be large enough to clarify changes due to using glycerol in diet. Therefore, for more accuracy research is also needed with greater numbers of animals to make a judgment about the effect of glycerol on reproductive performance of ruminants especially in goats (male and female).
2) Further studies required in the future to be establishing for the use of glycerol in rams for studding reproduction efficiency.
3) Researches required in the future confirming and explaining the results to studding yield and components of milk by using glycerol in dairy ewes.
4) Researches needed to apply for comparison during various periods (prepartum, postpartum and lactation through glycerol dietary in ruminant.
5) It is important to know glycerol effect on lambs growth during puberty and sexual maturity, and glycerol role on growth hormone.
6) To take into consideration metabolism study profile and glycerol effect on thyroid gland hormones.
7) Possibility using of glucoplastic substance or oleochemical products as energy source in ruminants diet and study the effects on reproduction and production.

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LIST OF PUBLICATIONS:

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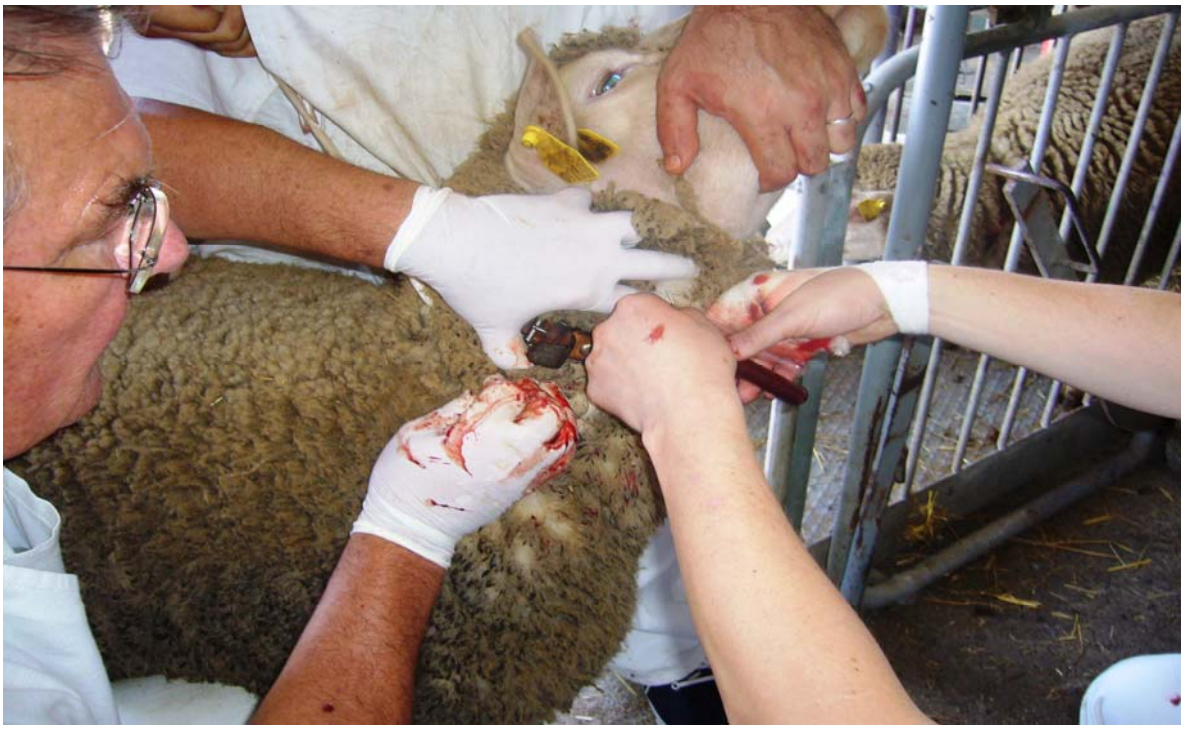
10. APPENDIX

Picture 1: Container with crude Glycerol (80%)

Picture 2: Meadow hay & Alfalfa hay



Picture 3: Blood collection from jugular vein

Picture 4: Blood samples

Picture 5: Trial animals (4th group, control)

Picture 6: Trial animals (2nd group)

Picture 7: Elisa apparatus (METERTECH ∑ 960)

Picture 8: Spectrophotometer apparatus (SCHOTT, Uviline 9400)

Picture 9: Haematology Analyzer apparatus (Abacus Junior vet.)