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OXIDATIVE STRESS DURING PREGNANCY AND LACTATION OF LOHI SHEEPS

DOCTORAL DISSERTATION

Menthor: Student:

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Novi Sad, 2021.

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Abstract	The objective of the present investigation was to evaluate the oxidative stress during pregnancy and lactation in Lohi sheeps reared in Lybia. Investigations were carried out on total 70 Lohi sheeps between 1.5 to 5 years of age. Total of 40 sheeps were sub-grouped into various stages of pregnancy along with non pregnant control group as follow: non pregnant (n=10), early (n=10), mid (n=10) and late pregnantcy (n=10), as well as milking stages such as lactation I (n=10), lactation II (n=10) and lactation III (n=10). Blood samples were collected from pregnant and lactating sheeps

during various pregnant and production stages, and preserved at -20 °C and further used for evaluating concentration of Total antioxidant status (TAS), Total oxidant status (TOS), Malondialdehyde (MDA), Superoxide Dismutase (SOD), Catalase (CAT), Vitamin E and C, Paraoxonase (PON-1), Arylestrase (Ary), Ceruloplasmin (Cp), Homocysteine (Hcy), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Gamma-glutamyl transferase (GGT), Glucose, Cholesterol, High-density lipoprotein (HDL-C), Low-density lipoprotein (LDL-C), Triglycerides (TG), Triiodothyronine (T_3), Thyroxine (T_4) and Cortisol. Statistical analysis was done by ANOVA and Duncan Multiple Range test. In pregnant sheeps TAS, TOS, MDA, SOD, CAT, PON-1, Ary, Hcy, AST, cholesterol, HDL, LDL, TG, T_3, T_4 and cortisol showed significantly different results. TOS, MDA, Ary, Hcy, cholesterol, TG and cortisol have been increased from non pregnant ewes and does to early, mid and late stage of pregnancy whereas, TAS, SOD, CAT, PON-1 and HDL-C showed a significant decrease with the progression of pregnancy. Moreover, AST and LDL-C have been increased from non pregnant to early and mid pregnant ewes, however their values were decreased in advance stage of pregnancy. T_3 values were increased with the progression of pregnancy in Lohi sheep whereas T_4 concentration was decreased from non pregnant ewes to early pregnant ones but increased during mid and late stages of pregnancy. In Lohi sheeps vitamin E, vitamin C, Cp, ALT, GGT and glucose gave significantly the same values during various stages of pregnancy. Irrespective of groups, overall mean values among various stages were significantly different for TAS, TOS, MDA, SOD, CAT, Ary, Cp, Hcy, AST, GGT, cholesterol, HDL, LDL, TG, T_3 and cortisol. During lactation, TOS, MDA, SOD, CAT, Cp, GGT, cholesterol, LDL-C, triglycerides, T_3 and T_4 were manifested significantly different results during various stages of lactation. In ewes, TOS, MDA, SOD, CAT, Cp and cholesterol showed their maximum concentration during lactation stage II; however, LDL-C and T_4 were giving the least values during this peak lactation period. Additionally, GGT, triglycerides and T_3 concentrations decreased from lactation I to III. The overall mean values for TAS, TOS, PON-1, Ary, Hcy, Cp, AST, GGT, cholesterol, LDL-C, triglycerides, T_3, T_4 and cortisol presented significantly different results. The present findings revealed that pregnancy and lactation were the most stressful and energy demanding physiological periods thus marked changes in prooxidant and antioxidant system along with higher strains in liver enzymes, hormones, glucose and lipid profile were observed.

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CONTENT

ABSTRACT

Aim of these research were to investigate the oxidative stress during pregnancy and lactation in Lohi sheeps reared in Lybia. Investigations were carried out on total 70 Lohi sheeps in age between one and a half to five years, respectively. Total of 40 sheeps were sub-grouped into various stages of pregnancy along with non pregnant control group as follow: non pregnant (n=10), early (n=10), mid (n=10) and late pregnantcy (n=10), as well as milking stages such as lactation I (n=10), lactation II (n=10) and lactation III (n=10). Blood samples were collected from pregnant and lactating sheeps during various pregnant and production stages, and preserved at -20 °C and further used for evaluating concentration of TAS, TOS, MDA, SOD, CAT, Tocopherol and Ascorbic acid, PON-1, Ary, Cp, Hcy, AST, ALT, GGT, Glucose, Cholesterol, HDL-C, LDL-C, TG, T_3, T_4 and Cortisol. Statistical analysis was done by ANOVA and Duncan Multiple Range test. In pregnant sheeps TAS, TOS, MDA, SOD, CAT, PON-1, Ary, Hcy, AST, cholesterol, HDL, LDL, TG, T_3, T_4 and cortisol showed significantly different results. TOS, MDA, Ary, Hcy, cholesterol, TG and cortisol have been increased from non pregnant ewes and does to early, mid and late stage of pregnancy whereas, TAS, SOD, CAT, PON-1 and HDL-C revealed a considerable reduction with ongoing gestation. Additionally, AST and LDL-C get increased from non pregnant to early and mid pregnant ewes, however their values were decreased in advance stage of pregnancy. T_3 values were increased with the progression of pregnancy in Lohi sheep whereas T_4 concentration was decreased from non pregnant ewes to early pregnant ones but heightened throughout middle and final gestation phases. Concerning Lohi sheeps vitamin E, vitamin C, Cp, ALT, GGT and glucose gave significantly the same values during various stages of pregnancy. Irrespective of groups, overall mean values among various stages were significantly different for TAS, TOS, MDA, SOD, CAT, Ary, Cp, Hcy, AST, GGT, cholesterol, HDL, LDL, TG, T_3 and cortisol.

During lactation, TOS, MDA, SOD, CAT, Cp, GGT, cholesterol, LDL-C, triglycerides, T_3 and T_4 were manifested significantly different results during various stages of lactation. In ewes, TOS, MDA, SOD, CAT, Cp and cholesterol showed their maximum concentration during lactation stage II; however, LDL-C and T_4 were giving the least values during this peak lactation period. Additionally, GGT, triglycerides and T_3 concentrations decreased from lactation I to III. The overall mean values for TAS, TOS, PON-1, Ary, Hcy, Cp, AST, GGT, cholesterol, LDL-C, triglycerides, T_3, T_4 and cortisol presented significantly different results. The present findings revealed that pregnancy and lactation were the most stressful and energy demanding physiological periods thus marked changes in prooxidant and antioxidant system along with higher strains in liver enzymes, hormones, glucose and lipid profile were observed.

1. INTRODUCTION

Free radicals are important unstable and reactive molecules which are formed as by-products of oxidation reduction reactions and are categorized as ROS and RNS [1]. Generally, development of ROS were was equivalent to removal of these species and animals were in the status of oxidative balance [2]. Actually, an inequity or imbalance could arise whenever, the formation of ROS increases or there was decrease in rate of their removal through antioxidant systems [3,4]. This condition is called oxidative stress [5]. Oxidative stress means status of oxidative overload, whether this term used for the cell, organ or organism [6].

Due to oxidative stress, not only the structure but functions of the lipids, proteins, nucleic acids and enzymes were damaged and consequently cause tissue damage [7]. The oxidative damage of lipids through ROS is normally produced during various physiological statuses in a balanced, suitable amount of these radicals and is an adaptive defense against stress [8]. However, lipid peroxidation in an excess is very critical and initiate self stimulating chain reaction and consequently released malondialdehyde (MDA) as degradation by-product and is key indicator of pro-oxidants and used for evaluation of oxidative stress [4,9].

Oxidative stress study is very imperative in evaluating homeostasis disturbances and production in farm animals [10]. It is immensely hazardous because does not reveal any symptoms and is identifiable with immense difficulty by various analysis methods of antioxidant defense elements and products of oxidative stress in terms of ROS [3,11]. Oxidative stress is a new field used for evaluation of metabolic imbalances in farm animals for better maintenance and production of these animals. Very few conditions or statuses have been studied for determining influence of oxidative stress in sheep were evaluated oxidative stress when homeostasis is disturbed [12].

There may be imbalance of oxidative status during various physiological conditions like pregnancy [13], parturition [14] and lactation [15]. Pregnancy is associated with dynamic fluctuations in metabolic activities resulted into enhanced basal oxygen consumption and energy requirement [16]. Not only the appropriate hormones required for establishment of placenta but surplus nutrients were also needed for development and growth of foetus [17]. Thus mother's reserves for nutrients were now mobilized to meet the demand and consequently formation of ROS were enhanced and both foetus and mother were facing oxidative stress [18]. Negative energy balance was evident during late pregnancy which consequently develops oxidative stress; enhanced lipid peroxidation and lowered activity of antioxidants [19]. National Research Council (NRC) was approved approximately 1.5 times more energy requirement of ewes for maintaining homeostasis in late pregnancy [20]. During parturition, mother's physical attempt for foetus removal and the action of hormones were the cause of metabolic imbalance and excess development of ROS. Increased demands for energy during early lactation would initiate oxidative reactions and electron flow increased thus induced ROS formation [21].

As the development of ROS were counterbalanced through antioxidant defense system [22]. Antioxidants are those substances which could reduce, delay or prevent the oxidative damage targeted upon biomolecules [23]. The antioxidant defense system includes enzymatic (SOD, catalase and GSHPx) and non enzymatic antioxidants (vitamin E, vitamin C and ceruloplasmin) [24]. Superoxide dismutase (SOD) is first line of defense against ROS and convert O_2^- to H_2O_2 further more catalse convert H_2O_2 into oxygen and water [25]. Vitamin E containing α-tocopherol and protects against lipid peroxidation by reacting with ROS [26]. Vitamin C or ascorbic acid can check

peroxidative process and also oxidized vitamin E [27]. Ceruloplasmin is an intravascular antioxidant and checks lipid peroxidation process and feed deficiency; its level was increased near parturition [28]. Paraoxonase-1 or arylesterase is associated in blood with high density lipoprotein (HDL) and hydrolyse organophosphates and aromatic carboxylic acids esters [29]. PON_1 protects HDL and LDL from oxidation [30].

To investigate redox status of the organism, it was recommended that there should be used at least 1 marker for oxidative damage (pro-oxidant) and 1 antioxidant marker [31]. The most efficient and successful method for measuring pro-oxidants and antioxidants equilibrium is through a single measure, total antioxidant status (TAS) and total oxidant status (TOS) [32].

Blood metabolic profile (BMP) was used for assessing metabolic status in veterinary clinical medicines [33]. It is a trustworthy tool to determine input and output ratio in terms of nutrient and production; maximum yield with minimum cost was obtained [34]. BMP includes the concept of diagnostic laboratory evaluation of certain components of the blood that predicted heath status of the animal with or without clinical symptoms. The BMP parameters in recent studies, includes glucose, cholesterol, HDL-C, LDL-C and triglycerides [35]. Glucose is the major energy metabolite needed for reproduction and production of animals [36]. Energy and nutrients requirement enhanced during pregnancy and lactation [37]. Cells of mammary gland have exploited almost 80% blood metabolites for production of milk [38]. There was increased serum lipid profile; enhanced cholesterol and triglycerides concentration because the target tissues have reduced receptiveness towards insulin along with enhanced fatty acids mobilization from adipose tissues during late pregnancy.

Physiological statuses (pregnancy and lactation) are the critical phase that influenced upon various indicators of blood [39]. Pregnancy and lactation causes infilteration mass in liver and damage hepatocytes and consequently these cells release liver enzymes like Aspartate transaminase (AST), Alanine aminotransferase (ALT) and Gamma-glutamyltransferase (GGT) [40]. AST, ALT and GGT were progressively increased during pregnancy and at parturition time, highest concentration was noted [40]. Ewes during lactation were shown enhanced ALT

activity as compared to dry ones [41]. Homocysteine (Hcy) is an amino acid and defend against oxidative stress through transsulfuration process. Hcy was studied for evaluating health status, feed deficiency; during pathological conditions and during various physiological conditions (pregnancy, lactation) [42].

Thyroid hormones, triiodthyronine (T_3) and thyroxine (T_4) are important for energy maintenance, thermoregulation and in reproduction for differentiation, development and growth [43]. T_3 and T_4 were shown an increased during late pregnancy than a decrease level during parturition and early lactating stage but again shown an enhancement during late lactation. Cortisol is oxidative stress indicator and its level differs during various stages of pregnancy and lactation [44].

For many centuries, sheep were used for obtaining milk, meat, skin, fiber, manure and also for various physical works in different conditions [45]. Some landless farmers in tropical arid areas kept sheeps for personal use and for sale. Sheep are very important due to their biological factors such as short generation interval, twinning, have short growth periods, and do not require much space.

Among various breeds of sheep, Lohi breed is famous for its high-quality meat along with high growth rate and maximum income is achieved through lamb production [46]. It is having large and massive body with average weight of 45-62 kg. Body colour is white with dark brown head but drooping ears and tail is heavy and small. Lohi sheep were given 40% of national sheep production [46].

Oxidative stress determination is best tool for improved reproductive performance in sheep [47]. As no significant information is available in the literature on the Lohi sheep [48] on the physiological biomarkers in relation to pregnancy and production at different stages, it is being hypnotized that these biomarkers during different stages of pregnancy and production will behave quite differently.

2. REVIEW OF LITERATURE

2.1. Free radicals

Any species or group of species with one or more unpaired electrons and can have its own independent existence is known as "free radical" [49]. These highly reactive and unstable free radical species donate or gain electrons from other molecules for the sake of pairing their electrons and can generate more stable species. Free radicals are generally produced as by-products of redox (oxidation-reduction) reactions and are categorized into two main groups: reactive oxygen species (ROS) and reactive nitrogen species (RNS) [50]. But generally, RNS like NO (nitroxide) are also recognized as ROS [51]. ROS perform as major important mediators in all the cell functions when produced in physiologically limited concentrations [52]. However, they provoke oxidative stress when formed in surplus quantity and can be the cause of cell as well as the tissue injuries [52].

Nitric oxide (NO) and nitrogen dioxide are common examples of reactive nitrogen species [53]. Through nitric oxide synthase (NOS) enzyme; conversion of L-arginine to L-citrulline will resulted into Nitric oxide (NO) [54]. NO being unpaired electron is behaving like highly reactive free radical and not only damages carbohydrates, nucleotides, proteins and lipids but along with other inflammatory mediators causes cell and tissue damage, low grade, inflammation and adhesions [54].

The three major significant types of ROS are: hydroxyl ($OH^{\bullet}$), superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) [3,4,55]. When leakage of electrons occurred from electron transport chain than there is production of superoxide radical and through the process of superoxide dismutation hydrogen peroxide was produced. The hydroxyl ion ($OH^{\bullet}$) is also extremely reactive and have ability to convert purines and pyrimidines and also can affect DNA strands resulting in DNA damage. Similarly, hydrogen peroxide radical was directly synthesized through oxidase enzymes [56].

Reactive oxygen metabolites (ROMs) having the ability to attack all of the major categories of biomolecules, however the most vulnerable class include lipids. More than 100 diseases were implicated through ROS [57]. Additionally they have more adverse effect in the physiological and pathological function in female reproductive tract. Different researchers have studied the impact of ROS in ovaries oviducts and embryos [58]. There is involvement of ROS in the modulation of all the aspects of physiology of reproduction such as oocyte maturation, ovarian steroidogenesis, role played by corpus luteum and also in luteolysis and also causes female infertility. ROS may also produce through embryonic metabolism and its surroundings [59].

2.2. Oxidative stress

Under physiological conditions, antioxidants cause inhibition of free radical generation and also prevention of substrate oxidation or can behave as scavengers and through the process of neutralization of free radicals, change them into molecules which are chemically stable [60].

A critical balance exists between generation of free radical and defence system through antioxidant. Oxidative stress occurs when there is inequity between oxidants and antioxidants in support of oxidants [61]. Oxidative stress can be described that metabolic condition of cell, tissue, organ or as an entire individual with oxidative overload. The occurrence of oxidative stress is dependent to that route from which ROS are generated. It can be sub-categorized as metabolic oxidative stress, environmental oxidative stress, photoxidative stress or nitrosative stress [61]. Metabolic oxidative

stress is dependent to activity of mitochondria since O_2 consumption is not absolutely match to the production of ATP [24].

Oxidative stress is sometimes useful to stimulate biological pathways at that time when counterbalancing by antioxidants and necessary to adapt the animals. Like it stimulate repairing and healing of tissues; therefore is not damaging at all time, but only in excess production [28]. Oxidative stress is tremendously hazardous since it did not reveal any signs and is identifiable with immense difficulty through some of the general analysis methods. This variation of oxidative balance causes an oxidative stress and cellular or tissue damage if it was not efficiently counter balanced by antioxidant systems and thus organisms are exposed to various degenerative diseases [44]. ROMs are generally produced during normal physiological functions but when produce in excess i.e. during strenuous activities and pathological conditions induces oxidative stress. Therefore free radical evaluation through oxidative stress will determine the organism condition[57].

During pregnancy, ewes having multiple fetuses are facing more strenuous metabolic stress and is the main reason of pregnancy toxaemia and they are influencing not only energy requirement through metabolism but also albumin and bilirubin that in turn affect antioxidant status [62]. This status is also affected by antioxidants provided through food [62].

During pregnancy, ROS are produced by foetus and mother for the growth of foetus in terms that ROS promote replication, differentiation and maturation of cells and organs [63]. Additionally, ROS are involved for implantation, development and defense of embryo in uterine infection, maintenance of pregnancy, birth process and steroidgenesis [63]. However, ROS when exceed can cause mother-foetus exchange by placenta degeneration, embryo resorption, disturbed growth and stillbirths. In a study of effect of antioxidant enzymes in sheep corpora lutea (CL) was studied and have resulted that through enhancement of these enzymes activities, luteolysis was rescued [64]. ROS are normally generated in steroidogenically healthy luteal cells and were protected from luteal steroidogenic activity and cellular integrity through these antioxidants [64].

Transition period is the risky period in terms of alterations in metabolic disturbances and energy homeostatsis due to enhancement of milk production.

Negative energy balance (NEB) was generally occurred during this period when animals passed from a stage of late pregnancy to early lactation due to striking augment of energy requirement. NEB requires interactions of metabolic fuels and its failure was faced by liver and adipose tissues. As a result of NEB, nonesterified fatty acids (NEFA) are produced in the liver and cause of excess ROS production and development of oxidative stress. Hence, antioxidative or prooxidative statuses along with metabolic profiles are a helpful means for evaluation of health and production status during the transition period [65].

2.3. Evaluation of oxidative stress

Numerous studies were performed to examine the oxidative stress parameters in ewes when homeostasis is changed [66]. Pregnancy and lactation are physiological conditions of the animals in which there is increased demand of energy and oxygen in all tissues specially placenta and foetus. Similarly, for milk production there required an adequate amount of energy thus enhances oxygen consumption. It is most likely to be happened that ROS and antioxidants might be related to various important processes like milk production [13,66].

There are four major steps for oxidative stress including ROS production, Antioxidant defense, oxidative damage and oxidative repairing [67]. The evaluation of oxidative stress due to shorter life span of ROS and repair system capability measurement *in vivo* are very challenging tasks. Consequently, researchers majorly focused on indirect OS biomarkers which detect damage to DNA, plasma membranes, proteins and lipids. Biologists have suggested that to evaluate ROS or redox status of an organism, there must be studied one marker for antioxidant defense and at least one marker for oxidative damage [67,68].

There are so many researches which proof that the main attacking sites of oxidative stress are the plasma membranes or intracellular organelles membranes where ROS reacted with unsaturated fatty acids and thus cause peroxidation of membrane lipids [4]. The targeted molecules for oxygen radicals are unsaturated fatty

acids which were mainly associated with the phospholipids of the membranes and as a result cause disarrangement of cell structure and its function [69]. Peroxidation of the membranes causes an outflow of cellular material and desiccation which ultimately cause cellular death. Polyunsaturated fatty acids produced lipid peroxides which are highly reactive and unstable and readily decomposed into a series of compounds like malondialdehyde (MDA). Lipid peroxides derived from polyunsaturated fatty acids are unstable and are decomposed to form a series of compounds, including malondialdehyde (MDA). MDA is used as a biomarker of pro-oxidants and readily used for oxidative stress evaluation [4,55].

Free radical production is accessed through plasma level of ROMs [70]. It is a term which is used for the calibration of not only superoxide anion and hydroxyl radical, but various non-radical oxygen derivatives like hydrogen peroxide and hypochlorous acid. A ROMs kit was discovered to evaluate plasma and different other body fluids oxidant status. The electron spin resonance was used to evaluate ROMs test and has been proved best “gold standard” for determining oxidative stress. Electron spin resonance has not been appropriate for usual evaluation of stress because of complexity and also technical expertise has required and not available in majority of laboratories [70]. The efficiently use of the ROMs kit in evaluating oxidative stress was reported in sheep [71].

To evaluate a dynamic balance between production of pro-oxidants and antioxidants, TAS has been applied. Whereas, oxidative stress index (OSi) was a standard measuring technique determined through ratio of Total oxidant status (TOS) to Total oxidant status (TAS) because the effects which were employed by all oxidants are additive [72]. TAS was considered very effective method for determining OS when used as a single measure and provides relevant information of pro-oxidants and antioxidants equilibrium.

Research aimed to evaluate lipid peroxidation intensity (LPI) and antioxidants status (TAC) in colostrums and milk in healthy cows was performed by several researchers [73]. Samples were collected after parturition immediately, 6 hours, 18 hours and 7 days gap. TAC concentration was enhanced from 6th hour to end of sampling time were shown highest. However, changes in LPI were vaguely changed with maximum values at 36 h. The ratio of TAC/LPI was gradually increased from 0h

to 7th day. However, TAC and LPI were not correlated positively. Therefore, LPI was remained stable for defense against ROS while TAS was changed [73].

Albera and Kankofer [73] studied for analyzing antioxidative or oxidative balance in colostrum, milk and blood during two lactation cycles in cows. Total of 19 healthy cows were observed for two years and samples were taken at 24, 48 hand 12 days. There were great changes observed for oxidative values in colostrum, milk and blood. TAC and GSH-Px were shown a decrease; however protein and lipid peroxidation have shown fluctuations within the lactation. When a comparison was made between two lactation cycles, lipid and protein peroxidation were changed in both the cycles; however GSH-Px has given opposite trend. These results were needed further work in terms of molecular and biochemical background [73].

Abuelo et al. [74] were conducted a study for evaluation of oxidative stress index (OSi) which was measured through pro-oxidants and antioxidants ratio during transition period in dairy cows. Cows were divided into four groups including prepartum (including parturition), postpartum, peak lactation and late lactation. The results were shown an increase in oxidative stress after parturition. Therefore, antioxidants supplementation was given 1month before parturition until peak of lactation [74].

Novac and Andrei [75] determined oxidants, nitric oxide and antioxidants levels in multiparous ewes along with lambs in extensive rearing setup. Samples were taken from both ewes and lambs (each=30) after day 1st , 5th and 10th of lambing for evaluating NOx, TOS, TAC and ferric reducing ability of plasma (FRAP). Nox were enhanced in lambs than their mothers at 5th and 10th day. TAC level was observed same in ewes and lambs at 1st and 5th day, however reduced at 10th day in lambs. TOS concentrations were significantly same after parturition in ewes but were highest at 1st day after parturition in lambs. FRAP values were enhanced at 10th day postpartum in ewes and were remained same in lambs. FRAP levels were higher in lambs at 1st and 5th day as compared to ewes. Thus it was concluded that lambs and ewes were shown different approach towards oxidants, antioxidants and nitric oxides and was playing vital role in lamb production [75].

Güneş et al. [76] analyzed PON1, oxidative stress index (OSi), TAS and TOS concentration in colostrum of Simmental cows. Total of 16 cows were chosen for 0-4

days samples in 5 groups (I, II, III, IV, V). Group V was having significantly high PON1 activity; however oxidative stress markers were higher in group IV. These findings suggest that oxidative stress was changed on daily basis in colostrum [76].

A study [77] was conducted for the evaluation of TAS and Selenium concentration in Holstein cows during different stages of lactation. Animals were divided into three groups according to lactation stage. Malondialdehyde (MDA), Total antioxidant status (TAS), selenium, glutathione peroxidase (GSH-Px) activity and thioredoxin reductase (TrxR) were parameters to be analyzed. Serum MDA, selenium and GSH-Px were showing an increase in early lactation than dry ones and peak lactating animals. However, TAS and TrxR have shown an opposite trend. These results confirmed that the early lactation is the period of greater risk by ROS and lipid hydroperoxides as serum MDA level was increased and TAS and TrxR were decreased that showed a lower antioxidant defense during the period [77].

2.4. Antioxidants

Antioxidants are actually those substances whenever exists in low level than an oxidizable substrate, than would cause to inhibit or delayed the process of oxidation of that substrate [78]. Antioxidants caused the reduction of hydrogen peroxide to water and alcohol [79]. The antioxidant system includes different categories of exogenous and endogenous components like enzymatic, non enzymatic antioxidants, vitamins and non vitamins etc [80].

1. Enzymatic antioxidants are natural antioxidants whose levels fluctuate due to fluctuations of ROS. These include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione peroxidase (GPx). SOD takes part in the catalytic reaction of superoxide anion (O^{-2}) to hydrogen peroxide (H_2O_2) to reduce the adverse effects of O^{-2}. GSH-Px destroys lipid hydroperoxides and hydrogen peroxide produced during physiological processes [81].

2. Non-enzymatic includes ceruloplasmin, melatonin and thiol antioxidants, ascorbate, urate, and beta carotene [82].

3. Vitamins includes vitamin A, C, E and non-vitamin includes polyphenols and carotenoids and they synergistically use for the neutralization of free radicals [83].

Measurement of different antioxidant molecule levels, or assays testing the cellular capacity to cope with an oxidative challenge, is also commonly applied. The most complex body antioxidant systems include non-enzymatic antioxidants which can be recognized as dietary supplements or synthetic antioxidants [84]. These includes vitamins and minerals like vitamin E and C, zinc, selenium, glutathione, carotene, beta carotene, taurine and hypotaurinen. Vitamin E is a fat soluble antioxidant with most active homologues α-tocopherol that reacted with ROS produce during oxidative stress and defends the cell membranes from lipid peroxidation. Vitamin E requirement increased during entire duration of pregnancy. Vitamin C or ascorbic acid is a water soluble antioxidant against ROS and defends DNA damage [84]. Vitamin C can be used like a chain breaking antioxidant which helps to inhibit the synthesis of the peroxidative process. Vitamin C also facilitate in the recycling of glutathione and oxidized vitamin E. Whereas, taurine, transferrin and hypotaurine are usually associated with tubal and follicular fluid resultantly defend the embryo from OS [85].

Ceruloplasmin is an acute phase protein which plays a vital role in serum transport of 90-95% Cu. It is synthesized in liver as a result of tissues damage or inflammation condition. Moreover, act as significant intravascular antioxidant and defend against lipid peroxidation. There was increased in lipid peroxidation and oxidative stress during physiological statuses like normal pregnancy and lactation consequently ceruloplasmin activity would also increase and the probable reason might be enhanced adrenal steroids concentration during stress [86]. Ceruloplasmin could act as an important indicator for food deficiency especially at the end of pregnancy. Copper is the main element for two enzymes which are acting as important antioxidants including ceruloplasmin and SOD [87].

Antioxidant condition of ewe's blood during pregnancy and lactation was discovered that the lipid peroxidation in red blood cells is decreased, while in blood plasma it is increased in the middle of pregnancy, reaching at the maximum shortly before the peak of progesterone production [88]. The activities of antioxidant enzymes are increased at the last of pregnancy, while the level of progesterone in blood is

maximal [88]. It is revealed that the antioxidant enzymes behavior attain a maximum during critical periods of pregnancy, providing the protection of the mother and the fetus against negative influence of free radicals [89]. The positive relationship between level of hormones and different parameters of antioxidant system in blood allows us to suppose that progesterone participate in the regulation of enzymatic part of the antioxidant system, whereas cortisol is concerned in the regulation of the non-enzymatic constituent of this system in ewes [90].

Erisir et al. [91] studied in Awassi sheep to assess antioxidant or oxidant profile through some parameters like MDA, CAT, GSH, GSH-Px concentrations before and different stages of pregnancy. Total of 20 healthy ewes and 2 rams were used for grouping. Samples of females were taken just before synchronization and at every 25th day during all the months of pregnancy. The MDA level was decreased in 2nd and 3rd month when compared to non pregnant sheep and sheep in 1st, 4th, 5th months of pregnancy; however GSH and GSH-Px were shown opposite trend during 2nd and 3rd months. Similarly, serum CAT concentration was decreased during 2nd and 3rd month of pregnancy. These findings in ewes were the indication of predisposition of oxidative stress during 2nd and 3rd month of pregnancy [91].

Studies on fat tailed sheep in female and males both for evaluating serum MDA, SOD, CAT and GSH which have shown oxidative stress level. They have resulted into significantly same values for all of these parameters of oxidative stress between sexes [92].

Research of antioxidant status of fat-tailed ewes 3 to 5 years old, for the duration of breeding and early-to-mid pregnancy under dry range conditions with supplementary feed, which are generally recognized as periods with low to moderate nutritional demands, was conducted as well. Activities of superoxide dismutase (SOD) and glutathione peroxidase (GPX) present in red blood cells in addition to the erythrocyte contents of thiobarbituric reactive substances (TBARS) were used to observe the oxidative status [93]. The results were compared, within a 120-day period, among a range of days of sampling (days 1, 7, 21 and 120) started with the introduction of rams into the ewe herd. The SOD and GPX activities significantly reduced and were related with significant raise in TBARS occurring as early as day 21, continuing less intensely to day 120. Ewes with different body conditions and ages were affected

equally. Fat tailed ewes might be suffered from oxidative stress throughout breeding and pregnancy and the breeding time might be a more demanding period than pregnancy.

Sakha et al. [94] have aimed to known the impact of pregnancy toxemia and consequent hyperketonemia on serum ceruloplasmin and copper level in ewes. Samples were taken from 5 ewes of 3-4 years age and 45-50 kg weight before and after induction of pregnancy toxemia. Serum ceruloplasmin and BHBA were significantly increased while glucose and copper levels concentration were reduced after the induction of pregnancy toxemia. It is concluded that ceruloplasmin is usually used for copper level evaluation but during last month care should be taken [94].

Evaluated fluctuations in some enzymes and other biochemical parameters in ewes with pregnancy toxemia was researched. The pregnancy toxemia in sheep is show to be an metabolic disorder which usually occured at the end of pregnancy near parturition and characterized by hyperketonemia. Pregnant normal and pregnant hyperketonemic ewes at their last month were selected and compared for serum evaluation of ceruloplasmin, NEFA, AST, ALT, glucose, cortisol, total protein and calcium. NEFA, ceruloplasmin and cortisol were increased significantly in hyperketonemic ewes while glucose and calcium have shown opposite trend. It was concluded that hyperketonemic ewes have shown hypoglycemia and hypocalcemia but hypercortisolemia in last gestation month. Enhanced ceruloplasmin concentration would have shown its importance during pathophsiological stressful condition [95].

Researchers have evaluated the change in markers of blood redox status during perinatal period and affect was studied before and after lambing as well as during lactation. Blood was taken from 12 ewes divided into seven groups including: before pregnancy; 1.5 months before pregnancy; 24.0 h before pregnancy; 2.0 h and 1day before parturition and 4^{th} and 8^{th} week of milking. Lipid peroxides, MDA, SOD, CAT, TAS, urea, uric acid creatinine and bilirubin were analysed. Redox status markers were similar in non pregnant and the pregnant sheep up to 1.5 month before parturition. These findings have concluded that there was homeostasis in redox status and ewes were free of oxidative stress during this period. Redox status was changed during perinatal period and thus remained at this enhanced level till 8^{th} week of lactation [96].

Pregnancy and health affect on oxidative stress in sheep have been studied . The sheep were grouped into high diet (concentrated diet) and low diets (natural forage). MDA, TAC, CAT and SOD were measured through blood. MDA was increased in pregnant animals whether the feed may be; however TAC was lowered in those pregnant animals given low diet. CAT was observed low in grazing pregnant animals while SOD was decreased independent to feed effect. Pregnancy included the most stressful phase especially for those facing low diets in arid saline in Egypt [97].

In addition to above mentioned antioxidants; some others antioxidants includes paraoxonase and arylesterase. Among the paraoxonases family (PON I-III), PON I is extensively studied especially in mammals. PON I is a glycoprotein of 43-45 kDa size and at first explained in 1953. PON I is a calcium-dependent esterase and is produced from liver while when present in serum it is associated with high density lipoprotein (HDL). In fact, the enzyme was initially known by organophosphate hydrolyse because of its most common substrate, paraoxon [98].

In veterinary sciences, PON I research was giving attention only in bovine, though now the interest of researchers was shown in different organisms like dogs, horses and cats. However, in sheep it still needs to be explored [98].

PON-I activity could be deliberated through spectrophotometer assays or through direct immunological procedure by the use of antibodies. PON I is a promiscuous enzyme due to having hydrolyzing abilities like paraoxonase activity (diethyl p-nitrophenyl phosphate, E600), the toxic oxon metabolite of parathion. Thus its name paraoxonase is derived from hydrolyzing paraoxon, arylesterase activity (phenyl acetate) or lactonase activity (5-thiobutil butyrolactone-TBBL). Other substrates like diazinon, chloropyrifos, soman or sarin in organophosphorus group included [99].

There was lowering of PON activity during pregnancy and lactation and it might be due to the reason of increased oxidative stress or decreased cholesterol and HDL concentration in blood or mobilization of fat and accumulation of triglycerides in liver thus also the reason of liver damage or the combination of all these factors. Similarly, periparturient is a period of evaluating oxidative stress disorders and antioxidant activity in the serum. Therefore PON I and arylesterase activities associated with HDL were used as indicator for the diagnosis of different disease during this period [99].

Serum PON I activity was measured during early lactation in two different groups of pregnant postpartum cows and non pregnant lactating cows. PON I was reduced after parturition in first group than second one. During postpartum period, total cholesterol, LDL-C and triglycerides were also shown a decrease; however significantly same results were shown by HDL-C. Thus PON I decrease was correlated with lipid metabolic changes and also due to lowering of antioxidant capacity during this early postpartum period [100].

2.5. Serum enzymes

Pregnancy and lactation are physiologically very challenging stage as for the energy and nutritional need and metabolic disturbances due to production of stressors in organisms. Animals usually suffered from negative energy balance (NEB) due to glucose utilization and non-esterified fatty acids (NEFA) accumulation produced by hepatocytes in response to decrease in VLDL. Due to infiltration of fatty liver and destruction of hepatoocytes caused cell membrane damaged and released increased amount of liver enzymes like AST, ALT, GGT and LDH. The increased amount of these enzymes was the sign of cell necrosis and soft tissue damage and missing vitamin E [101].

Aspartate transaminase (AST) was formerly named as serum glutamic oxaloacetic transaminase while alanine transaminase (ALT) as serum glutamic pyruvic transaminase. Another enzyme present in liver is Gamma glutamyl transferase (GGT). AST and ALT are involved in gluconeogenesis through displacement of amino group from aspartate and alanine to α-ketoglutarate and resultantly oxaloacetate and pyruvate are formed along with glutamate. ALT and GGT is usually present in liver cells, however AST in liver cells, erythrocytes, skeletol and heart muscles. These enzymes are used as an important marker of hepatic devastation [102].

El-Sherif et al. [47] evaluated blood metabolic profile during pregnancy and lactation stasuses in Barki ewes. AST, ALT enzymes, total protein, albumin, globulin, creatinine, urea, hemoglobin, mean corpuscular hemoglobin concentration (MCHC)

and packed cell volume percent (PCV%) were the parameters under evaluation. Dry ewes have constant values for all the parameters; however pregnant animals have shown increased values and maximum level at lambing. Lactating ewes have shown decreased PCV level and this might be reason of decreased hemoglobin at 4th week. During lactation, ewes were shown increased ALT, glucose, MCHC, total protein, urea and creatinine when a comparison was made with dry ones. There was decreased in globulin level but increased Adenine/Guanine ratio and AST concentration as compared to dry ewes [47].

Tibbo et al. [103] studied three different indigenous sheep breeds. They have researched the influence of age, breed and sex on various parameters like AST, ALT, ALP and acid phosphatse. No significant variations were observed for all these parameters in all these breeds. Thus concluded that breed, sex and age have no effect on the above parameters and would need further studies in this field [103].

Luz et al. [104] investigated lactation effect on oxidative or antioxidative status, composition and production of milk and biochemical metabolites in blood and milk in sheep. Group A containing 7 ewes were selected and at 1 and 7 days were induced 0.5mg/kg (body weight) estradiol and 1.25mg/kg (body weight) progesterone. Additionally, at 11 and 40 days they were also induce 250mg/kg (body weight) bovine somatotropin and at 19, 20 and 21 days 16mg/day dexamethasone. Whereas, group B having 5 ewes were taken as control and samples from both the groups were drawn for analysis. The hormones induced lactation while production of milk was 79% low as compared to control group. Group A have decreased levels for milk fat, total solids and protein than group B. ALT, GGT and albumin concentration were much higher while decreased urea concentration was noted in group B than group B. Serum antioxidant FRAP were enhanced and ROS decreased in experimental group than control one. FRAP were shown high in group A as compared to group B. These findings were resulted that treatment for induction of lactation in blood and milk metabolites were not successful [104].

2.6. Serum biochemical profile

Homocysteine (Hcy) is an amino acid having sulphur as main element synthesized during methionine metabolic pathway. Hcy could be changed into cysteine through transsulfuration reaction and cysteine through reactions would further produced glutathione (GSH). Therefore, it was proved that Hcy transsulfuration process would help to defend against oxidative stress. It was noted that folate or cobalmine deficiency would affect Hcy metabolism both in mothers and foetus which was the cause of early pregnancy loss, placental damage and preeclampsia. In foetus, hyperhomocysteinemia would cause loss of growth, neural tube disorders, abortion and premature birth [105].

Oxidative stress was playing a contributable role during homocysteine metabolism. Different researches have done for taking an account of homocysteine either due to protein or vitamin deficiencies or during various pathological statuses and sometimes through genetic basis. Due to protein deficiency in rats during gestation and lactation caused oxidative stress and hypohomocysteinaemia in females and offsprings too. High energy requirements during lactation would increase oxidative stress and is directly affected level of animal production [106].

Piccione et al. [107] evaluated the effects of stage of lactation to homocysteine as well as overall oxidative stress in ewes. Healthy ewes at same production and lactation stage were selected and analysed for antioxidant barrier (oxy-adsorbent) and thiol antioxidant barrier, ROMs and serum homocysteine. In ewes to estimate the stages in the milking period were used and antioxidant barrier (Oxy-adsorbent), thiol antioxidant barrier, reactive oxygen species (ROMs) and homocysteine (Hcy) were investigated. There were shown significantly different results for all these parameters throughout the study period would suggest high level of oxidative stress late lactation phase. Consequently, oxidative stress determination and its impact on homocysteine would be important marker for health status during lactation period and for best management practices [107].

Kanakkaparambil et al. [108] studied the effect of homocysteine and vitamin B-complex supplied upon fertility and pregnancy stability especially on folliculogenesis in ewes. Samples were taken from 76 sheep in 3 groups. There was decrease in insulin

that would utilize to increase antral follicles through FSH stimulation through this supplementation; inspite of decreased live weight in 1st group. There was shown an increase in homocysteine concentration and consequently would enhance proliferation of granulosa cells, estradiol production and FSH transcript expression. It was concluded that this supplementation would enhance fertility [108].

The evaluation of different blood indicators includes blood metabolic profile (BMP) through standards methods. Major BMP includes the biochemical and hematological parameters. Due to increased energy demands, the alteration of metabolites has been taken during various physiological stages like pregnancy and lactation especially during their early stages. Likewise, secretary cells in mammary glands would consume almost 80% circulating metabolites during lactating phase Serum chemistry is also beneficial during various diagnosis studies [109].

Yokus et al. [110] evaluated the influence of physiological statuses and season on serum chemistry and vitamins in ewes. The ewes were divided into 2 groups of mated and non-mated ewes and samples were drawn at early and mid pregnancy, during lactation and during dry season. Cholesterol, ALT, T_4, total protein, globulin, creatinine and uric acid were significantly altered during various reproductive phases but significantly same in seasons. Triglycerides, VLDL, Vitamin A and E, AST, ALP, LDH, T_3, urea and creatine kinase were altered during various reproductive phases as well as during various seasons. However, glucose, folate and c-glutamyl transpeptidase concentrations were significantly same for seasons as well as physiological statuses. The present findings were interpreted that serum biochemistry alterations should be considered during reproductive statuses and seasonal variations to reduce economic losses [110].

Piccione et al. [107] analyzed various biochemical parameters during different stages of pregnancy and lactation in ewes. Samples were drawn from 10 ewes before pregnancy, during the pregnancy, 2 week after parturition and during of lactation and dry period. Total blood lipids were shown a significant increase during pregnancy and early lactation whereas; total cholesterol and triglycerides were observed an opposite behavior as compared to dioestrus. These results were shown a significant alteration during pregnancy, post-partum and lactation stages.

Beura et al. [111] determined influence of balanced high concentrate feed on growth, hematological and various metabolites during lactation in ewes and lambs. There were two sampling groups of same parity (20 animals/group; uniform body weight) and were given either grazing (control) or concentrated feed of 200g/day (experimental) to each individual early in the morning before grazing for the duration of first 2 months of lactation. Experimental group has shown increased body weight starting from 2nd to 5th fortnights to all studied period. There was shown enhanced values of serum glucose concentration in ewes of group 2 than group 1 at 4th fortnight and in lambs this enhanced level was observed at their age of 12th fortnight. Total leukocyte count, hemoglobin and mean cell hemoglobin (MCHC) values were shown fluctuations in both the groups after parturition at 4th fortnight. MCHC concentration was shown higher in experimental group than control group. It was concluded that concentrated feed could augment mothers and lambs body weight and also hematological parameters [111].

2.7. Plasma hormones

In animals, there are tremendous metabolic modifications taking place during the physiological conditions like pregnancy and lactation and the stress enhanced especially during last 2-3 months of pregnancy [112]. Thyroid hormones could have considerable role for the determination of metabolism of lipids and carbohydrates during the transition period. Thyroid hormones, triiodthyronine (T_3) and thyroxine (T_4) were well known that were playing a significant role during differentiation and growth. Serum T_3 and T_4 concentrations were elevated during pregnancy than significantly decreased during parturition and early lactation than increased during late lactation stage. The increased level of T_3 and T_4 during late pregnancy was due to estrogen levels. Cortisol is biomarker of stress and its level is varying during various stages of gestation, parturition and lactation. There was enhancement of cortisol with the progression of pregnancy was due to increased adrenocorticotrophic hormone that cause release of glucocorticoids from adrenal gland [112].

Kachhawa et al. [113] aimed to determine base line values for hemato-biochemical profile and hormonal activities in sheep. Samples were selected from government farm and animals were grouped into three including: anestrus, pregnant and post-partum phases. Hematological parameters including hematocrit and RBCs were high during pregnant ewes during breeding season when compared to other reproductive statuses. Platelets count was lowered while MCHC were observed enhanced during post-partum than other phases. During post-partum phases, biochemical parameters including serum cholesterol, Ca, K and Mg were noted enhanced while significantly lowered concentration were seen for total protein, globulin and Na. As endocrine profile was concerned, higher concentration of T_4 and estrogen concentration during pregnancy and serum TSH and progesterone during gestation were observed. It was concluded that there were significant variations in blood metabolites and hormonal profile during various physiological statuses in sheep [113].

Belkacem et al. [114] studied about the fluctuations in hormonal profile, key metabolites and blood parameters during estrous and various stages of pregnancy in ewes. Samples were taken from non pregnant but cyclic ewes, early, mid and late pregnant stages. There were shown significant high MDA, GSH, progesterone, HDL. Cyclic animals were shown high levels of glucose, insulin, T_3, T_4 and SOD. However, cholesterol, LDL, VLDL, triglycerides and leptin were significantly same. These findings were concluded that reproduction have great impact on hormonal and metabolic metabolism and might be used for the evaluation of oxidative stress and energy disturbing metabolic diseases [114].

3. AIM OF THE RESEARCH AND HYPOTHESIS

3.1. Objectives of the research

- To establish the health status of sheep through oxidant, antioxidant and homocysteine parameters
- To determine effect of oxidative stress during different stages of pregnancy and lactation
- To investigate possible changes of the serum biomarkers activity during different stages of pregnancy and production
- To examine the relationship between biomarkers and others serum biochemicals such as HDL, LDL, T_3 and T_4

4. Materials and methods

Biological experiment with sheeps was performed following the EU legislation and principle of the Three Rs within Directive 2010/63/EU.

4.1. Selection of sheeps

All selected sheeps for the investigations was healthy and care in completely with all animal welfare standards. Early pregnant as well as lactating animals were provided with Lucien, hay, maize silage and concentrate (crude protein, CP); however, late pregnant animals were offered grass hay, wheat straw, corn maize, alfalfa, soyabean and wheat flour. The animals were given fresh clean water twice a day. All the animals were vaccinated as per schedule and treated with anti-helminthics after 6 months.

Clinically normal and healthy Lohi sheep (n=70) aged 1.5-5 years were selected for the present study. Each group of animals was sub grouped into pregnant and lactating stages. Pregnant group included control group of Non Pregnant, Early pregnant (40-50 days), Mid pregnant (60-90 days) and Late pregnant (100 days-onward) animals. Whereas, lactating period would comprise of Early, Peak and Low lactation stages as Lac I (30 days), Lac II (60 days) and Lac III (90-onward).

4.2. Sampling of blood

Veterinarians were engaged for sampling of blood during the studied period. Blood sampling was done early in morning time and samples have been taken aseptically from jugular vein from each animal at different stages of their pregnancy and production in volume of 5 ml for EDTA-K2 and 5 ml for clotting.

Blood samples with anticoagulant and without anticoagulant were used for biochemical analysis.

The samples without anticoagulant were centrifuged at 167 × g for 15 minutes and serum was harvested and preserved at -20 °C in small aliquots till further analysis in accreditated laboratory.

4.3. Biochemical analysis

4.3.1. Total antioxidant status (TAS; mmol Trolox Equiv./L)

Different antioxidants in a coordinated fashion give better defense against ROS, instead of any of compound act separately. Total antioxidant status was consisted of various compounds and their systematic metabolic interactions. Antioxidant activity which we use as a term is occasionally used as antioxidant capacity; however the first one is used to determine the constant rate of particular single antioxidant against provided free radical. While total antioxidant capacity is the analysis of moles for a present radical scavenged by test solution and is independent to any antioxidant activity of such particular molecule in reaction mixture. Therefore total antioxidant capacity shows good index for antioxidant status when a comparison was made for evaluation of antioxidant property of a single molecule.

In the present assay, 2, 2'-azino bis 3-ethylbenzo thiazoline 6-sulphonate (ABTS) has been oxidized to $ABTS^{+}$ when present in acidic medium in the presence of H_2O_2. While ABTS dissolved in acetate buffer (30mM/L) at 3.6 pH, it has been oxidized into

most stable $ABTS^{+}$ which showed by dark green color. After the addition of dil. acetate buffer of high pH (0.4M/L; 5.8 pH), color was slowly bleached. In this mixture, after the addition of serum sample the antioxidants of the serum was accelerated the bleaching rate up to a level of equivalent concentration. By means of using spectrophotometer, bleaching process was checked and this rate was inversely proportional to total antioxidant capacity (TAC) of the sample. This reaction rate has been monitored by Trolox (an analogue of Vit. E). It was the most extensively used TAC assay standard (measured in mM Trolox equivalent/L). The linearity of the assay was 6.0 mmol trolox equivalent/L and shown by less than 3% precision error.

To evaluate TAC a semi auto-analyzer Biosystem (BTS-330) was used for spectrophotometer study. From spectrophotometer, Monochromatic light (660nm wavelength) was selected and warmed the selected filter for about 5 min. 200 µl Reagent was mixed with 5 µl serum/samples/standards. The first reading was taken before mixing of reagent I with II and it was used as a blank. After that 20 µl reagent II was added to above mixture. This mixture was now incubated at 37 °C for 5min. and second absorbance was read. By means of using standard curve against the standards, the delta absorbance was used to calculate TAC.

4.3.2. Total oxidant status (TOS; µmol H_2O_2 Equiv./L)

The oxidants which were present in the samples caused an oxidization of *O*-dianisidine complex into ferric ion. With the use of glycerol in this mixture, reaction could be accelerated. The Xylenol orange which is present in acidic medium along with ferric ions made the complex coloured. Spectrophotometer was used to study the color intensity; it was depicted the direct measurement of sample's oxidant molecules. H_2O_2 was used for calibration purpose and results were shown as µmol H_2O_2 Equiv. L^{-1}. The assay was sensitive at 1.13 µmol H_2O_2 Equiv. L^{-1} along with precision rate less than 3% and linearity was shown up to 200 µmol H_2O_2 Equiv. L^{-1}.

Serum samples in aliquote of 35 µl were added into 225 µl reagent I and first absorbance was taken immediately. In this serum sample and reagent I mixture, 11 µl reagent II was mixed and after 4 minutes final absorbance was read. Bichromatic wavelength containing main/primary 560 nm and 800 nm secondary/differential

wavelengths was used for absorbance. The actual concentration of µmol H_2O_2 Equiv. L^{-1} was calculated from standard curve by using delta change in absorbance.

4.3.3. Malondialdehyde (MDA; mmol/mL)

MDA was measured by Lipid Peroxidation (LPO; µmol/L) method. Kits (Abacum, UK) were provided with components along with directions of their use and storage.

LPO assay FTS reagent 1, LPO assay FTS reagent II, Lipid hydroperoxide standard, LPO Assay Extract R and LPO assay Triphenylphosphine were prepared by using 1 vial of each component and stored as per instructions. Before initiating LPO estimation method, Lipid hydroperoxidase was extracted to form chloroform. 1ml final volume of assay was attained in each test tube. According to instructions in the protocol, standards were run with each one test simultaneously and in triplicate.

4.3.4. Superoxide dismutase (SOD; µ/mL)

Method of Kostadinović et al. [3] was used for determination of serum SOD activity.

Xanthine-xanthine oxidase was employed to produce superoxide flux. Nitroblue tetrazolium (NBT) was resulted and has been proceed as an indicator for production of superoxide. Degree of inhibition of reaction unit of enzyme was used to determine SOD activity and that would cause 50% inhibition for reduction of NBT. Results were expressed in µ/mL.

To prepare 0.052 Sodium pyrophosphate buffer 2.32 g Sodium pyrophosphate was dissolved in 80 ml distilled water and by adding 1N HCL pH was adjusted to 8.3 than raised the volume upto 100 ml through distilled water and it would preserve at 4 °C.

For the preparation of 186 µM Phenazinemethosulphate solution, 5.697924 mg phenazinemethosulphate was dissolved in 100 ml of distilled water. For the preparation of 300 µM Nitrobluetetrazolium solution, 24.5292 mg

nitrobluetetrazolium was dissolved in 100 ml of distilled water. To prepare 780 µM NADH solution, 51.74754 mg NADH was dissolved in 100 ml of distilled water.

Total of 500 µl aliquots serum was added into 500 µl ethanol and 300 µl chloroform and centrifuged for half an hour at 18,000 × g. 900µL SOD reagent was prepared by mixing 0.1 mmol/l xanthine + 0.1 mmol/l ethylene diamine tetra acetic acid (EDTA) + 25 mmol/l nitro blue tetrazolium (NBT) + 50 mg serum (BSA) and 40 mmol/l Na_2CO_3 maintained at pH 10.2. The reagent was mixed with 50 µL of supernatant removed after centrifugation and incubation was done at 25°C for 20 min. By adding 1 mL $CuCl_2$ (0.8 mmol/l), the reaction has been stopped and absorbance of samples has read at 560 nm. Percent inhibition was calibrated through formula:

$$\% \text{ inhibition} = (A_{blank} - A_{sample}) / A_{blank} \times 100\ \%$$

4.3.5. Catalase activity (CAT; KU/L)

A spectrophotometer assay based on H_2O_2 was used to analyze catalase activity through Kostadinović et al. [3] methodology.

1.0 ml substrat**e** was prepared by mixing of 65 µmol/ml H_2O_2 dissolved in 60 mmol/l sodium potassium buffer (pH 7.4). Incubation of 0.2 ml serum along with 1.0 ml substrate has been done at 25 C^0 for 1 min. Linearity of serum catalase was up to 100 KU/L. But in a case when catatlase activity increased from 100 KU/L, dilution of phosphate buffer from 2 to 10 fold was done and assay has been repeated. Under these circumstances, 1 unit catalase produced 1 µmol/ml H_2O_2/min.

By the addition of 1.0 ml of 32.4 mmol/l ammonium molybdate $[(NH_4)_6 Mo_7O_{24}.4\ H_2O]$ reaction has been stopped by production of yellow complex and this complex was read at 405nm against blank 3.

Following formula was used for calculation.

$$\text{Serum catalase activity (KU/L)} = \frac{\text{A (sample) - A (blank 1)}}{\text{A (blank 2) - A (blank 3)}} \times 271$$

4.3.6. Vitamin E (Vit. E; µmol/L)

Method of Rutkowski and Grzegorczyk [115] has been used for the evaluation of vitamin E.

4.3.7. Vitamin C (Vit. C; µmol/L)

Method of Rutkowski and Grzegorczyk [115] has been used for the evaluation of vitamin C concentration.

4.3.8. Paraoxonase activity (PON-I; U/min/mL)

Paraoxonase enzyme has following phenotypes i.e. PON-I, PON-II and PON-III and of all these PON-I is more active. PON I hydrolysis several types of organophosphates like paraoxon and aromatic esters like phenyl acetate, additionally lipid peroxidation products and also caused to reduce their accumulation. The rate of paraoxon enzymatic hydrolysis into p-nitrophenol was used as estimation for PON I activity. By means of spectrophotometer the color formed because of p-nitro phenol production was determined. Primary hydrolysis rate or sensitivity of hydrolysis was constant until 5 min.

4.3.9. Arylesterase activity (Ary; KU/min/L)

Another aromatic esterase is arylesterase which is an isoenzyme of paraoxonase phenylacetate was used as a substrate in reaction mixture for evaluation of arylesterase activity Turk et al. [116]. This phenylacetate was changed into phenol in the presence of arylesterase in mixture and rate of its formation revealed direct index for enzyme activity. Stock solution has been prepared by means of mixing

phenylacetate with a solution 40% methanol. Reaction mixture was made by mixing of following chemicals.

4.3.10. Ceruloplasmin activity (Cp; U/L)

The oxidase potential would determine the ceruloplasmin activity and for this purpose ortho-dianisidine dihydrochloride was used as a substrate. In the presence of enzyme ceruloplasmin and oxygen, the sample was turned yellowish brown. But due to acid formation the reaction was stopped and constant reddish purple color was formed in solution that maximum absorption wavelength of 450 nm wavelength and linearity would be determine up to 400 U/L with a 4.2% CV.

4.3.11. Total homocysteine (Hcy; µmol/L)

Total Homocysteine concentration in the serum was measured through microtiter plate assay. Polyethene culture tubes were used to prepare serum samples, after the addition of reducing agent tris (2-carboxyethyl) phosphine hydrochloride (TCEP) the protein bound Hcy was reduced to free Hcy which was consequently changed into S-adenosyl-L-homocysteine (SAH) due to the presence of SAH-hydrolase. It would finally quantitate by means of using horseradish peroxidase (HRP-SAH) competitive assay (Diazyme, Cat. No. DZ012A; Diazyme Laboratories).

4.3.12. Aspartate aminotransferase (AST; U/L)

Through the use of calorimetric method kit (Randox, Laboratories Limited, Ardmore, Diamond Road, Crumlin, Company, Antrim, UK, Lot No. 128011) the serum samples of AST in vitro was determined quantitatively. The concentration of AST was determined by observing concentration of oxaloacetate hydrozone along with 2, 4-dinitrophenyl-hydrazine which was formed by the reaction catalyzed by AST. When the absorbance of sample was under 0.17, we have maintained the linearity and coefficient of variance was observed less than 10%.

4.3.13. Alanine aminotransferase (ALT; U/L)

Through calorimetric method kit (Randox, Labotaries Limited., Ardmore, Diamond Road, Crumlin, Company, Antrim, UK, Lot No. 128011) the quantitative in vitro in the serum samples of ALT was determined. The concentration of ALT was determined by observing concentration of pyruvate hidrozone along with 2, 4 - dinitrophe nyl hydrazine which was formed by the reaction catalyzed by ALT. When the absorbance of sample was under 0.5, we would maintain the linearity and coefficient of variance was observed less than 10%.

4.3.14. Gamma glutamyltransferase (GGT; U/L)

Enzymatic calorimetric assay as described by Szasz [117] was used for determination of serum samples of GGT *in vitro*.

4.3.15. Glucose (mg/dL)

Spectrophotometer (screen master; 35510) along with Fluitest Glu Biocon (Lot no. H. 265) was used for the determination of glucose concentration. Enzymatic calorimetric test based upon Triender-reaction was used for measuring serum glucose.

4.3.16. Cholesterol (mg/dL)

Enzymatic calorimeter testing kit (Fluitest, Biocon DiagnosemittelGmbH & Co. 34516 Vohl-marienhagen, Germany, Lot No. N593) has been used for determination of cholesterol concentration. In this assay, cholesterol esterase and cholesterol oxidase were the enzymes used for enzymatic determination of cholesterol. The measurement limit was 3-800 mg/dL and coefficient of variance (C.V.) was less than 10% .

4.3.17. High density lipoprotein cholesterol (HDL; mg/dL)

From cells to liver reverse cholesterol transport was carried out by means of high density cholesterol (HDL). Fuitest HDL-Cholesterol kit (Biocon, Diagnosemittel GmbH & Co. 34516 Vohl-marienhagen, Germany, Lot No. Q115) was used to determine HDL-C. Magnesium chloride and phosphotungstic acid (provided in the kit as reagents) were precipitated chylomicrons, LDL (low density lipoprotein) and VLDL (very low density lipoprotein). The supernatant containing HDL-C was now centrifuged and measured by enzymatic assay like that of total cholesterol. Minimum measurable HDL-C conc. which could be differentiated ranges from zero to 3.0 mg/dl while coefficient of variance was below 10%.

4.3.18. Low density lipoprotein cholesterol (LDL; mg/dL)

By subtracting HDL from total cholesterol, LDL was calculated.

$$\text{LDL (mg/dL)} = \text{Total cholesterol (mg/dL)} - \text{HDL (mg/dL)}$$

4.3.19. Triglycerides (TG; mg/dL)

A kit of Triglyceride liquicolormono GPO-PAP method kit (Human Gesellschaft fur Biochemica and Diagnostica GmbH Max-Planck-Ring Wiesbaden, Germany, Lot No. 09014) has been taken for evaluation of triglycerides. The hydrolysis reaction of triglycerides by lipase enzyme was used to determine triglycerides. Hydrogen peroxide along with 4-aminoantipyrine and 4-chlorophenol were converted into quinoenimine by catalysis of enzyme peroxidase. Quinoenimine was used as an indicator in this catalytic reaction. The linearity of the test for triglycerides concentration was 1000 mg/dl and coefficient of variance was below 10%.

4.3.20. Triiodothyronine (T_3; ng/mL)

T_3 Elisa kit (Biocheck Inc., Foster City, CA-94404, USA, Lot No. RN - 41498) was used for evaluation of serum T_3 conc. quantitatively. In this EIA, Kit was provided with secondary antibody (goat anti-mouse IgG) coated microtiter well strips. Competitive ELISA has been done by addition of proper quantity of sample along with mouse anti T_3 antibody and T_3 conjugated with an enzyme named radish peroxidase. There was a competition between T_3 conjugated with peroxidase and T_3 present in the sample because of restricted sites on anti-T_3 antibody. Change in color was observed when an enzyme substrate TMB was added and T_3 conc. of sample has been inversely proportional to intensity of color change. The lowest amount of T_3 which could be measured was 0.2 ng/ml and CV has been below 10%.

4.3.21. Thyroxine (T_4; µg/dL)

By means of using T_4 EIA test kit (Biocheck Inc., Foster City, CA-94404, USA, Lot No. RN - 41498) has been used for quantitative evaluation of serum T_4 conc.

Competitive ELISA has been done by addition of proper amount of sample along with mouse anti T_4 antibody and T_4 conjugated peroxidase. There was a tough competition between T_4 conjugated with peroxidase and T_4 in the sample because of restricted sites on anti-T_4 antibody. Change in color was observed when chromagen substrate mixture was added and T_4 concentration in sample was inversely proportional to intensity of color change. The lowest amount of T_4 which could be measured was 0.4 µg/mL.

4.3.22. Cortisol (ng/mL)

A microplate EIA kit provided by Accubind (Monobind Inc., Lake Forest, CA-92630, USA, Lot No. 3625-300) was used for quantitative evaluation of serum cortisol concentration.

Competitive ELISA has been used which has shown a tough competition between enzyme conjugated antigen (kit) with sample's native antigen because of restricted sites on biotinylated antibodies. The binding of enzyme conjugated antigen (kit) with antibodies was observed by enzyme activity and was inversely proportional to sample's native antigen. The lowest amount of T_4 which could be measured was 0.4 to 95 µg/dl and regression coefficient was 0.98 along with 0.25 µg/dL sensitivity.

4.4. Statistical analysis

Data obtained will be subjected to two-way analysis of variance (ANOVA) techniques. The data were analyzed by using STATISTICA 13 statistical software. Duncan Multiple Range test will be applied to explain significance difference between different phases of pregnancy and production.

5. RESULTS AND DISCUSSION

5.1. Parameters during pregnancy

Total antioxidative status of Lohi sheep was observed by two way ANOVA and the results are presented in Table 1. Sheeps as well as their pregnant stages have showed sigificant ($P \leq 0.01$) differences. Similarly, interaction between these groups with stages was also showing significant different results. Lohi sheep were followed a decrease in TAS concentration from non pregnant to all subsequent stages though only non pregnant and advance pregnant ewes were different statistically. Lohi sheep did show higher values of TAS concentration throughout all the stages. Overall mean TAS concentration in non pregnant as well as throughout all the pregnant stages was significantly different ($P \leq 0.01$) and followed similar pattern of significant decrease from non pregnant up to advance pregnant stages. Overall mean TAS concentration also exhibited significantly different results (Table 2).

Two way ANOVA has been applied to observe the difference involved in the groups of sheep for total oxidant status (TOS) (Table 3). Groups of sheeps, in various pregnant stages and their interaction were showing different results ($P \leq 0.01$) significantly. Mean ± SE of total oxidant status concentration observed an increase from first stage of non pregnant animals to advance pregnancy in the ewes; however significantly ($P \leq 0.01$) different results were observed by mid and advance pregnant animals in ewes. Irrespective to groups, overall mean values among stages were significantly different; a significant increase in TOS was observed from non pregnant to all subsequent pregnant stages. The overall mean TOS concentration between the groups also revealed significantly different results (Table 4).

Table 1. Evaluation of Total Antioxidant Status (TAS; mmol Trolox Equiv./L) of Lohi sheep during different stages of pregnancy by means of Analysis of variance

Source of Variation	Degree of Freedom	Sum of Squares	Means Squares	F-Value
Groups (G)	1	0.440	0.440	72.232**
Stages (S)	3	6.844	2.281	374.892**
G × S	3	0.315	0.105	17.245**
Error	72	0.438	0.006	
Total	39	8.037		

**Significant - P ≤ 0.01

Table 2. Evaluation of Mean ± SE of Total antioxidant status (TAS; mmol Trolox Equiv./L) of Lohi sheep during different stages of pregnancy

Stages	Lohi Sheep
Non Pregnant (N.P)	1.93±0.02 [a]
Early Pregnant (E.P)	1.73±0.02 [b]
Mid Pregnant (M.P)	1.67±0.01 [b]
Advance Pregnant (A.P)	1.22±0.01 [d]
Overall Means	1.64±0.04

a-d; Table showing same alphabets do not differ significantly (P ≤ 0.01).

Findings have shown a significant decrease in Total Antioxidant Status (TAS) concentration from non pregnant to advance pregnant stages in sheep, however, significant results were given by non pregnant and late pregnant ewes [118]. A new methods to develope and evaluate total antioxidant status (TAS) in animals was develepoed such as total antioxidant capacity (TAC). Similar results were obtained by others that the Total Antioxidant Capacity (TAC) value was highest in dry ewes and decreased with progression of pregnancy [118].

As pregnancy being a stressful physiological condition and this stress in turn caused an increase in cortisol concentration. The enhancement of cortisol with the advancement of pregnancy was confirmed by our results of cortisol during pregnancy and this might be the reason for increased TOS but decreased TAS level because

cortisol promoting lipolysis [119]. The decrease in TAS might be coincident with the absence of vitamins and mineral supplementation, i.e. exogenous antioxidants, during the pregnancy.

Similar results in the reduction of TAS during pregnancy were confirmed by different researchers [118]. Similarly, it was observed decrease TAS activity just before parturition. TAC was decreased during pregnancy as compared to non pregnant groups. Similarly "Oxidative Stress Index" was described as the ratio of ROS/TAS increased from dry to pregnant animals throughout all stages of pregnancy; thus confirmed our results for TOS increase and TAS decrease.

Table 3. Evaluation of Total oxidant status (TOS; µmol H_2O_2 Equiv./L) of Lohi sheep during different stages of pregnancy by means of Analysis of variance

Source of Variation	Degree of Freedom	Sum of Squares	Means Squares	F-Value
Groups (G)	1	0.081	0.081	17.871**
Stages (S)	3	10.048	3.349	742.251**
G × S	3	0.254	0.085	18.732**
Error	72	0.325	0.005	
Total	39	10.707		

**Significant - P ≤ 0.01

Table 4. Evaluation of Mean ± SE of Total oxidant status (TOS; µmol H_2O_2 Equiv./L) of Lohi sheep during different stages of pregnancy

Stages	Lohi Sheep
Non Pregnant (N.P)	0.48±0.01 ^{c}
Early Pregnant (E.P)	0.52±0.01 ^{c}
Mid Pregnant (M.P)	0.64±0.01 ^{b}
Advance Pregnant (A.P)	1.23±0.04 ^{a}
Overall Means	0.72±0.05

a-c; Table showing same alphabets do not differ significantly (P ≤ 0.01).

Serum MDA level of Lohi sheep was studied by two way ANOVA and Table 5 has been presented these results. Ewes with various pregnant stages exhibited significantly ($P \leq 0.01$) different results. Likewise a significant difference was also observed between groups of both all stages. In Lohi sheep serum malondialdehyde concentration did show a significant ($P \leq 0.01$) increase from non pregnant to advance pregnant stages in a sequence. Irrespective to groups, overall mean malondialdehyde values among various stages were also increased significantly from non pregnant animals to advance pregnant stages progressively. Results for overall mean malondialdehyde concentration in sheep were also significantly different (Table 6).

In order to observe difference in serum SOD concentration in sheep throughout various pregnancy stages, two way ANOVA was used (Table 7). Significantly different ($P \leq 0.01$) results were observed in sheep throughout various stages of pregnancy. In Lohi sheep mean ± SE of serum superoxide dismutase concentration was different significantly within non pregnant animals along with the animals at various pregnant stages; significantly high values were observed in non pregnant animals while a progressive decrease was noted in early, mid and advanced pregnant animals. Lohi sheep did show significantly high superoxide dismutase values in early, mid and advance pregnant stages. Overall mean superoxide dismutase values in non pregnant animals and among various stages of pregnancy irrespective to sheep groups were also following same pattern of significant decrease from non pregnant sheep through all the stages of pregnancy. Overall mean superoxide dismutase concentration was statistically significant (Table 8).

A significant increase in Total Oxidant Status (TOS) concentration was observed from early pregnant to advance pregnant Lohi sheep and non pregnant ewes have shown significantly different values as observed during early pregnant animals. Our results were in consistent with others studied TOS level in pregnant and non pregnant females and found a significant increase in TOS concentration in pregnant ones [120].

As pregnancy being a stressful physiological condition and this stress in turn caused an increase in cortisol concentration. The enhancement of cortisol was confirmed by our results of cortisol during pregnancy and this might be the reason for increased TOS but decreased TAS level with the advancement of pregnancy. The decrease in TAS may be coincident with the absence of vitamins and mineral

supplementation, i.e., exogenous antioxidants, during the pregnancy. This was further confirmed within studies of the impact of nutrition in sheep pregnancy and found lowering of TAC level especially in animals given low energy diets; thus facing more oxidative stress [121].

Table 5. Evaluation of Malondialdehyde (MDA; mmol/mL) of Lohi sheep and during different stages of pregnancy by means of Analysis of variance

Source of Variation	Degree of Freedom	Sum of Squares	Means Squares	F-Value
Groups (G)	1	5.703	5.703	283.119**
Stages (S)	3	25.685	8.562	425.021**
G × S	3	1.312	0.437	21.710**
Error	72	1.450	0.020	
Total	39	34.150		

**Significant - P ≤ 0.01

Table 6. Evaluation of Mean ± SE of Malondialdehyde (MDA; mmol/mL) of Lohi sheep during different stages of pregnancy

Stages	Lohi Sheep
Non Pregnant (N.P)	0.79±0.03 ^{ef}
Early Pregnant (E.P)	1.75±0.06 ^{cd}
Mid Pregnant (M.P)	2.11±0.08 ^{b}
Advance Pregnant (A.P)	2.60±0.05 ^{a}
Overall Means	1.81±0.11

a-f; Table showing same alphabets do not differ significantly (P ≤ 0.01).

In present study, Malondialdehyde concentration significantly increased from non pregnant to early, mid and advanced pregnant animals. Our results were in consistent with oters found an increase in MDA concentration with the advancement of pregnancy in sheep. The present findings were in consistent with the results found an increase in lipid peroxidation marker MDA with the advancement of pregnancy.

The likely cause of this rise in MDA concentration could be due to overproduction of free radicals (ROS) and thus induction of lipid peroxidation. The other reason for steady increase of MDA concentration with the progression of pregnancy was related with decreased levels of total antioxidant capacity (TAC), antioxidant enzymes i.e. catalase (CAT) and superoxide dismutase (SOD). This is also proved through our findings of decreased level of TAC, SOD and CAT enzymes with the advancement of pregnancy.

Table 7. Evaluation of Superoxide dismutase (SOD; µ/mL) of Lohi sheep during different stages of pregnancy by means of Analysis of variance

Source of Variation	Degree of Freedom	Sum of Squares	Means Squares	F-Value
Groups (G)	1	0.564	0.564	16.000**
Stages (S)	3	35.076	11.692	331.399**
G × S	3	1.111	0.370	10.494**
Error	72	2.540	0.035	
Total	39	39.292		

**Significant - P ≤ 0.01

Serum superoxide dismutase concentration was significantly decreased from non pregnant animals throughout different stages of pregnancy. Our results were in accordance with others reported a decrease in SOD activity from non pregnant ewes to the end of pregnancy i.e., first day before lambing. It has been showed a predisposition to oxidative stress in second to third months of pregnancy. Our results have shown great changes in redox status indicators signifying increase oxidative stress. While reactive oxygen species i.e. superoxide anion radical were formed in the organism, SOD transformed these into hydrogen peroxide through dismutation

process. Consequently this decrease in the concentration of lipid peroxides is probably linked to a reduction in SOD activity.

Our results were consistent with the results wich indicated a decrease in SOD concentration in pregnant sheep irrespective to high or low energy diets. Another study in ewes also proved the present findings of decreased SOD concentration with the progression of pregnancy.

Table 8. Evaluation of Mean ± SE of Superoxide dismutase (SOD; µ/mL) of Lohi sheep during different stages of pregnancy

Stages	Lohi Sheep
Non Pregnant (N.P)	3.35±0.06 [a]
Early Pregnant (E.P)	2.85±0.07 [b]
Mid Pregnant (M.P)	2.40±0.08 [c]
Advance Pregnant (A.P)	1.78±0.08 [de]
Overall Means	2.60±0.10

a-e; Table showing same alphabets do not differ significantly (P ≤ 0.01)

To see the difference in Lohi sheep for catalase activity, two way ANOVA was used; results were given in Table 9. Sheep groups in various pregnant stages as well as the interaction between groups × stages have been given highly significant (P ≤ 0.01) results. In Lohi sheep, non pregnant as well as all the pregnant stages were showing significantly (P ≤ 0.01) different results for serum catalase concentration; a significant high value of catalase activity was observed in non pregnant ewes that gradually decreased with the progression of pregnancy i.e. lowest value was noted in advance pregnant ewes. Overall mean catalase activity among various stages irrespective to groups was found a significant decreased value from 1st non pregnant stage to the last advance pregnant stage. Similarly, overall mean catalase concentration in sheep groups was also shown significantly different results (Table 10).

To observe the difference between Lohi sheep for serum vitamin E concentration, two way ANOVA was used and Table 11 was shown these results. Sheep groups as well as the interaction between the groups and stages were not differing significantly. However the results for different stages of pregnancy did show a significant difference.

In the Lohi sheep Mean ± SE of vitamin E concentration has shown significantly similar results; highest values were shown by non pregnant animals while insignificant decrease was observed in early and mid pregnancy and non significant increase during advance pregnant stage. Significantly similar results were observed in an interaction between these groups; within non pregnant animals and throughout all progressive pregnant stages. However, irrespective to groups, overall mean vitamin E concentration among various stages was shown significantly ($P \leq 0.01$) different results at various stages in the groups; highest vit. E value was observed in non pregnant animals that showed a significant decrease in early and mid stages of pregnancy but again an increase in vitamin E value was seen during advance pregnancy. Overall mean vitamin E concentration between groups was also shown significantly same results (Table 12).

Table 9. Evaluation of Catalase (CAT; KU/L) of Lohi sheep during different stages of pregnancy by means of Analysis of variance

Source of Variation	Degree of Freedom	Sum of Squares	Means Squares	F-Value
Groups (G)	1	1.070	1.070	30.698***
Stages (S)	3	19.052	6.351	182.277**
G × S	3	0.545	0.182	5.217**
Error	72	2.509	0.035	
Total	39	23.175		

**Significant - $P \leq 0.01$

Catalase concentration significantly decreased from non pregnant to different stages of pregnancy in sheep. Others found consistent results like ours by showing decreased catalase activity from non pregnant to early, mid and late pregnant stages in ewes [122]. Another study of effect of pregnancy and nutrition in pregnant sheep resulted into a decreased CAT level [123]. According to our findings, a decrease in antioxidant enzymes was observed and it might be due to the fact that superoxide radical was converted into hydrogen peroxide and oxygen by means of SODs, while the

CAT and peroxidases converted hydrogen peroxide into water. Thus, both of these toxic species, hydrogen peroxide and superoxide radical transformed into water.

Table 10. Evaluation of Mean ± SE of Catalase (CAT; KU/L) of Lohi sheep during different stages of pregnancy

Stages	Lohi Sheep
Non Pregnant (N.P)	3.30±0.07 [a]
Early Pregnant (E.P)	2.84±0.11 [b]
Mid Pregnant (M.P)	2.43±0.09 [c]
Advance Pregnant (A.P)	1.80±0.03 [d]
Overall Means	2.59±0.1

a-d; Table showing same alphabets do not differ significantly (P ≤ 0.01).

Production of free radicals was a necessary event during aerobic metabolism and cells can counterbalance their non toxic effects with metabolic strategies like antioxidant defense system. Non enzymatic agents like ceruloplasmin, vitamin C, and vitamin E were acting as antioxidants. Present study did not reveal any significantly different results for Vitamin E concentration in sheep groups throughout all the stages of pregnancy.

Other research confirmed our results that serum vitamin E levels give non significant differences between the pregnant and non pregnant groups of ewes. Our results were consistent with studies of the effect of vitamin E supplementation in ewes and found that control group and ewes with 2 or less than 2 lambs gave significantly similar results.

Table 11. Evaluation of Vitamin E (Vit. E; µmol/L) of Lohi sheep during different stages of pregnancy by means of Analysis of variance

Source of Variation	Degree of Freedom	Sum of Squares	Means Squares	F-Value
Groups (G)	1	0.001	0.001	0.463 NS
Stages (S)	3	1.096	0.365	200.101**
G × S	3	0.006	0.002	1.077 NS
Error	72	0.131	0.002	
Total	39	1.234		

**Significant - P ≤ 0.01 NS - Non-Significant

Table 12. Evaluation of Mean ± SE of Vitamin E (Vit. E; µmol/L) of Lohi sheep during different stages of pregnancy

Stages	Lohi Sheep
Non Pregnant (N.P)	1.57±0.01 NS
Early Pregnant (E.P)	1.38±0.02 NS
Mid Pregnant (M.P)	1.26±0.01 NS
Advance Pregnant (A.P)	1.41±0.02 NS
Overall Means	1.40±0.02

NS - Non-Significant

Serum vitamin C concentration of Lohi sheep were determined through ANOVA (two way) and Table 13 showed these results. Results for sheep groups and G × S interaction have not exhibited any statistically significant difference. Although in sheep, various pregnancy stages have been shown significant different (P ≤ 0.01) results. Lohi sheep groups in non pregnant as well as at different stages of pregnancy showed significantly same results for serum vitamin C concentration. Vitamin C level in ewes and does revealed non-significant results; a non significant decrease was observed from 1st stage of non pregnant animals to early pregnant ones after that an increase was seen in mid stage than again a decrease has been observed during advance pregnancy. Overall mean vitamin C values in sheep groups demonstrated significantly same results (Table 14).

Table 13. Evaluation of Vitamin C (Vit. C; µmol/L) of Lohi sheep during different stages of pregnancy by means of using Analysis of variance

Source of Variation	Degree of Freedom	Sum of Squares	Means Squares	F-Value
Groups (G)	1	0.008	0.008	0.118 ^{NS}
Stages (S)	3	32.283	10.761	154.407**
G × S	3	0.557	0.186	2.663 ^{NS}
Error	72	5.018	0.070	
Total	39	37.866		

**Significant - P ≤ 0.01 NS - Non-Significant

Table 14. Evaluation of Mean ± SE of Vitamin C (Vit. C; µmol/L) of Lohi sheep during different stages of pregnancy

Stages	Lohi Sheep
Non Pregnant (N.P)	5.37±0.11 ^{NS}
Early Pregnant (E.P)	4.87±0.05 ^{NS}
Mid Pregnant (M.P)	6.55±0.09 ^{NS}
Advance Pregnant (A.P)	5.10±0.11 ^{NS}
Overall Means	5.47±0.11

NS - Non-Significant

Vitamin C is one of the powerful reducing agent and radical scavengers; it has an antioxidant function through destruction of a variety of free radical directly or indirectly by reducing oxidized vitamin E in the membrane [124].

In present findings, vitamin C concentration did not show any significant difference through various stages of pregnancy. Our results were consistent with other results that Vitamin C maintained at a same level during pregnancy and perinatal period in ewes [125]. Similarly, it was revealed significantly the same results in the biochemical and hematological parameters in the does supplemented with vitamin C as well as in control ones. It has been found that vitamin C concentration decreased with the advancement of pregnancy in sheep [126].

Two way ANOVA was used to analyze the difference in serum paraoxonase concentration for sheep during different stages of pregnancy and results have been represented in Table 15. Sheep groups as well as their various pregnant stages along with groups × stages interaction have shown statistically significant results (P ≤ 0.01). In Lohi sheep, Mean ± SE of PON-I concentration was given significantly (P ≤ 0.01) different results; highest values were shown by non pregnant animals that have shown a significant decrease in early, mid and advance pregnant animals subsequently. Similarly, irrespective to sheep groups, overall mean PON-I concentration were also followed a significant decrease from non pregnant sheep to subsequent stages of pregnancy. Overall mean values between groups were however appeared significantly the same (Table 16).

Table 15. Evaluation of Paraoxonase (PON-I; U/min/mL) of Lohi sheep during different stages of pregnancy by means of using Analysis of variance

Source of Variation	Degree of Freedom	Sum of Squares	Means Squares	F-Value
Groups (G)	1	4977.013	4977.013	360.544**
Stages (S)	3	17052.137	5684.046	411.763**
G × S	3	649.937	216.646	15.694**
Error	72	993.900	13.804	
Total	39	23672.988		

**Significant - P ≤ 0.01

Table 16. Evaluation of Mean ± SE of Paraoxonase (PON-I; U/min/mL) of Lohi sheep during different stages of pregnancy

Stages	Lohi Sheep
Non Pregnant (N.P)	213.20±1.17 [a]
Early Pregnant (E.P)	199.70±1.19 [b]
Mid Pregnant (M.P)	178.00±1.33 [c]
Advance Pregnant (A.P)	171.10±1.89 [d]
Overall Means	190.50±2.78

a-d; Table showing same alphabets do not differ significantly (P ≤ 0.01).

PON-I is acting as antioxidant enzymatic defense against lipid hydroperoxides and lipid peroxides in low density lipoprotein (LDL) [116]. According to our results, there was a significant decrease in paraoxonase concentration with the progression of pregnancy in sheep groups; thus non pregnant animals had maximum value of paraoxonase in our results. However, information about serum PON-I and Arylesterase activity in veterinary medicine remained to be rare and there would scarce knowledge about relationship between PON-I and arylesterase activity during reproductive performance as well as during lactation in sheep [108].

To see the difference between Lohi sheep for arylesterase activity, two way ANOVA was used and Table 17 was presented the results. Significantly ($P \leq 0.01$) different results were observed for Lohi sheep groups, their various pregnant stages as well as their interaction between groups and stages. Mean ± SE of arylesterase concentration in Lohi sheep was significantly different ($P \leq 0.01$) not only during non pregnant stage but also throughout all the observed stages of pregnancy; lowest value has been observed in non pregnant animals that was increased significantly in early, mid and advanced pregnant animals respectively.

Table 17. Evaluation of Arylesterase (Ary; KU/min/L) of Lohi sheep during different stages of pregnancy by means of using Analysis of variance

Source of Variation	Degree of Freedom	Sum of Squares	Means Squares	F-Value
Groups (G)	1	816.003	816.003	233.028**
Stages (S)	3	4468.284	1489.428	425.340**
G × S	3	116.434	38.811	11.083**
Error	72	252.125	3.502	
Total	39	5652.847		

**Significant - $P \leq 0.01$

An interaction between the groups was significantly different during all the animals whether non pregnant, early pregnant, mid pregnant or advance pregnant ones. In a comparison between groups of sheep for various stages of pregnancy irrespective to groups, overall mean arylesterase concentration has exhibited significantly different

results in non pregnant and throughout various stages of pregnancy; Ary values were increased significantly from non pregnant to advanced pregnant stages in a sequence. The overall mean arylesterase concentration between these groups revealed statistically significant difference (Table 18).

Table 18. Evaluation of Mean ± SE of Arylesterase (Ary; KU/min/L) of Lohi sheep during different stages of pregnancy

Stages	Lohi Sheep
Non Pregnant (N.P)	53.75±0.57 [d]
Early Pregnant (E.P)	58.70±0.80 [c]
Mid Pregnant (M.P)	63.25±0.62 [b]
Advance Pregnant (A.P)	74.65±0.86 [a]
Overall Means	62.59±1.29

a-d; Table showing same alphabets do not differ significantly (P ≤ 0.01).

In our results, arylesterase activity was increased significantly from non pregnant to early, mid and late pregnant stages progressively in ewes.

Serum ceruloplasmin concentration in Lohi sheep was determined by using ANOVA (two way) and Table 19 has been shown the results. Sheep groups pertaining various pregnant stages were statistically different (P ≤ 0.01). However, interaction between groups and stages have not shown significant results. Serum Cp concentration was not given significantly different results in sheep. Non pregnant ewes and does having lowest value for Cp; the value increased non significantly to early, mid and advanced stages in sheep; however in does from mid to advance pregnant stage a non significant decrease in ceruloplasmin concentration was noted. Irrespective to groups, overall mean Cp concentration among various stages was significantly (P ≤ 0.01) different when compared non pregnant animals with mid and advance pregnant ones; however various pregnant stages among each other showed significantly the same results. Similarly, overall mean ceruloplasmin concentration between the groups was statistically different (Table 20).

Table 19. Evaluation of Ceruloplasmin (Cp; U/L) of Lohi sheep during different stages of pregnancy by means of using Analysis of variance

Source of Variation	Degree of Freedom	Sum of Squares	Means Squares	F-Value
Groups (G)	1	1914.435	1914.435	8.102**
Stages (S)	3	4958.418	1652.806	6.995**
G × S	3	970.166	323.389	1.369 NS
Error	72	17012.949	236.291	
Total	39	24855.968		

**Significant - P ≤ 0.01 NS - Non-Significant

Table 20. Evaluation of Mean ± SE of Ceruloplasmin (Cp; U/L) of Lohi sheep during different stages of pregnancy

Stages	Lohi Sheep
Non Pregnant (N.P)	119.60±0.82 NS
Early Pregnant (E.P)	123.85±0.66 NS
Mid Pregnant (M.P)	130.80±0.72 NS
Advance Pregnant (A.P)	144.20±0.81 NS
Overall Means	129.61±1.54

NS - Non-Significant

The present results were showing a non significant increase in serum ceruloplasmin concentration in non pregnant animals and throughout all the stages of pregnancy in sheep. Our results were confirmed by others where cerulolplasmin concentration was showing highest values in the pregnant ewe than non pregnant ones [127]. Ceruloplasmin is a vital intravascular antioxidant and controls lipid peroxidation. Furthermore, adrenal steroids are involved in stress-induced increase in ceruloplasmin activity. On the last week before parturition, oxidative stress level and lipid peroxidation increases in the pregnant animal.

Copper mineral is the vital component in two of these enzymes which are necessary for immune response; ceruloplasmin and copper/zinc-superoxide dismutase.

Total homocysteine concentration in serum of sheep has been analyzed by means of using two way ANOVA and Table 21 presented these results. Sheep groups through various pregnancy stages and groups × stages interaction exhibited significantly different ($P \leq 0.01$) values. Mean ± SE of serum Hcy concentration in Lohi sheep groups was significantly different in non pregnant stage and throughout all the stages of pregnancy; the lowest values were observed in non pregnant animals while a significant increase was observed in early, mid and late pregnant animals respectively. In Lohi sheep, homocysteine concentration did show much high values during all stages of pregnancy. Overall mean Hcy concentration was also observed significantly different in sheep groups (Table 22).

Table 21. Evaluation of Homocysteine (Hcy; µmol/L) of Lohi sheep during different stages of pregnancy by means of using Analysis of variance

Source of Variation	Degree of Freedom	Sum of Squares	Means Squares	F-Value
Groups (G)	1	3458.450	3458.450	866.568**
Stages (S)	3	17333.575	5777.858	1447.732**
G × S	3	2539.675	846.558	212.118**
Error	72	287.350	3.991	
Total	39	23619.050		

**Significant - $P \leq 0.01$

Table 22. Evaluation of Mean ± SE of Homocysteine (Hcy; µmol/L) of Lohi sheep during different stages of pregnancy

Stages	Lohi Sheep
Non Pregnant (N.P)	37.20±0.39 [d]
Early Pregnant (E.P)	42.10±0.43 [c]
Mid Pregnant (M.P)	54.20±0.79 [b]
Advance Pregnant (A.P)	89.10±1.24 [a]
Overall Means	55.65±3.27

a-d; Table showing same alphabets do not differ significantly ($P \leq 0.01$).

Our results have shown a significant increased homocysteine concentration from non pregnant ewes to early, mid and advance pregnancy progressively. Our results were confirmed by other study in ewes; ovarian response was improved for given methyl-deficient diet along with increased homocysteine concentrations [128]. The reason for Hyper-homocysteinemia might be shown by those animals with missing enzymes for folate/methionine metabolism, and/or whose diets were deficient in B vitamins i.e. vitamin B12 and folate.

Serum Aspartate transaminase concentration of Lohi sheep has been observed by two way ANOVA (Table 23). Sheep groups, various pregnancy stages along with G × S revealed different ($P \leq 0.01$) results statistically. An increase in AST values was noticed from 1st stage of non pregnant animals up to mid pregnant ones; however a decrease was seen in the animals during advance pregnancy. Mid and advance pregnant ewes have shown significant different results whereas does have shown significantly same results during all the stages. It has shown that overall mean AST values among various stages irrespective to groups gave significantly ($P \leq 0.01$) different results can be predicted that they were following the same pattern of an increase from non pregnant up to mid pregnancy stages than a significant decrease was noted during advance stages. Overall mean AST concentration was also significantly different between groups (Table 24).

Table 23. Evaluation of Aspartate aminotransferase (AST; U/L) of Lohi sheep during different stages of pregnancy by means of using Analysis of variance

Source of Variation	Degree of Freedom	Sum of Squares	Means Squares	F-Value
Groups (G)	1	6863.512	6863.512	1374.994**
Stages (S)	3	2687.337	895.779	179.455**
G × S	3	2134.638	711.546	142.547**
Error	72	359.400	4.992	
Total	39	12044.887		

**Significant - $P \leq 0.01$

Table 24. Evaluation of Mean ± SE of Aspartate aminotransferase (AST; U/L) of Lohi sheep during different stages of pregnancy

Stages	Lohi Sheep
Non Pregnant (N.P)	19.35±0.43 [c]
Early Pregnant (E.P)	22.00±0.47 [c]
Mid Pregnant (M.P)	45.85±0.96 [a]
Advance Pregnant (A.P)	36.60±0.90 [b]
Overall Means	30.95±1.77

a-c; Table showing same alphabets do not differ significantly (P ≤ 0.01).

To evaluate the variations of ALT concentration among various pregnant stages in sheep, two way Analysis of variance was applied (Table 25). Results for sheep groups and the interaction between groups × stages were not statistically significant. However, animals through various pregnant stages in sheep groups did show statistically different (P ≤ 0.01) results. Mean ± SE of ALT concentration was given significantly same results in ewes and does among all the stages of gestation. ALT concentration was showing non significant results; first a non significant increase was observed from non pregnant animals up to mid pregnant ones; however a non-significant decrease in advanced pregnant stage was found in the groups. Overall mean ALT concentration for both the groups was given significantly the same results (Table 26).

Table 25. Evaluation of Alanine aminotransferase (ALT; U/L) of Lohi sheep during different stages of pregnancy by means of using Analysis of variance

Source of Variation	Degree of Freedom	Sum of Squares	Means Squares	F-Value
Groups (G)	1	10.878	10.878	1.988 [NS]
Stages (S)	3	2152.684	717.561	131.153**
G × S	3	10.984	3.661	0.669 [NS]
Error	72	393.925	5.471	
Total	39	2568.472		

**Significant - P ≤ 0.01 NS - Non-Significant

Table 26. Evaluation of Mean ± SE of Alanine aminotransferase (ALT; U/L) of Lohi sheep during different stages of pregnancy

Stages	Lohi Sheep
Non Pregnant (N.P)	21.60±1.13 NS
Early Pregnant (E.P)	24.80±0.51 NS
Mid Pregnant (M.P)	35.05±0.84 NS
Advance Pregnant (A.P)	26.70±0.73 NS
Overall Means	27.04±0.89

NS - Non-Significant

Gamma glutamyl transferase concentration was evaluated by using two way ANOVA for Lohi sheep and their results were given in Table 27. The results between the groups were statistically significant ($P \leq 0.05$). Mean ± SE of serum gamma-glutamyl transferase level has not been given any significant difference in sheep Šgroups. In Lohi sheep GGT concentration has not demonstrated significantly different results; a non significant decrease was observed from non pregnant to early pregnancy than there was an increase during mid pregnancy than a decrease was observed again during advanced pregnancy. However, results for overall mean GGT concentration between the groups was observed to be significantly different (Table 28).

Table 27. Evaluation of Gamma-glutamyl transferase (GGT; U/L) of Lohi sheep during different stages of pregnancy by means of using Analysis of variance

Source of Variation	Degree of Freedom	Sum of Squares	Means Squares	F-Value
Groups (G)	1	2163.200	2163.200	4.981*
Stages (S)	3	787.513	262.504	0.604 NS
G × S	3	2915.625	971.875	2.238 NS
Error	72	31270.150	434.308	
Total	39	37136.488		

*Significant - P ≤ 0.05 NS - Non-Significant

Table 28. Evaluation of Mean ± SE of Gamma-glutamyl transferase (GGT; U/L) of Lohi sheep during different stages of pregnancy

Stages	Lohi Sheep
Non Pregnant (N.P)	25.15±0.42 NS
Early Pregnant (E.P)	19.60±0.72 NS
Mid Pregnant (M.P)	30.30±14.41 NS
Advance Pregnant (A.P)	14.30±0.45 NS
Overall Means	22.34±3.6

NS - Non-Significant

In recent study Gamma-Glutamyl Transferase concentration gave significantly similar results in sheep through all the stages of pregnancy. In sheep, a non significant decrease in GGT level was observed from non pregnant to early pregnant ewes than GGT increased in mid pregnancy; however a decrease in GGT level was observed again in late pregnant stage. Our results were consistent with others observed non significant results for GGT concentration in dry sheep as well as during different pregnant stages.

Others have also confirmed our results of non significant change in GGT between non pregnant and pregnant sheep [129].

Serum glucose concentration was measured for Lohi sheep groups through applying two way Analysis of variance and the results were shown by Table 29. Results for groups and interaction between groups × stages has not given significantly different results. However, various pregnant stages did show significant difference (P ≤ 0.01). Serum glucose level was not given statistically different results in 1st stage of non-pregnant sheep as well as throughout all the stages of pregnancy; however a non significant increase from 1st stage of non-pregnant animals to early and mid pregnant ones was noted that was again shown a decrease in advanced stage of pregnancy. Overall mean glucose values were found significantly different in non pregnant animals and among all the pregnant stages; highest value was shown by mid pregnant animals while lowest value by non pregnant animals. These mean values have predicted the same pattern followed by each group individually. Overall mean glucose concentration revealed significantly the same results in both the groups (Table 30).

Table 29. Evaluation of Glucose (mg/dL) of Lohi sheep during different stages of pregnancy by means of using Analysis of variance

Source of Variation	Degree of Freedom	Sum of Squares	Means Squares	F-Value
Groups (G)	1	11.858	11.858	3.349 NS
Stages (S)	3	1887.129	629.043	177.633**
G × S	3	0.419	0.140	0.039 NS
Error	72	254.970	3.541	
Total	39	2154.375		

**Significant - P ≤ 0.01 NS - Non-Significant

The present study has observed significantly the same results for glucose concentration throughout all the stages of pregnancy in sheep. Glucose was increased non significantly from non pregnant to early and mid pregnancy than a non significant decrease was observed during late pregnancy. Others also confirmed our results that glucose level was not affected through various physiological statuses including all the stages of pregnancy and lactation.

Sheep are usually protected from hypoglycaemia by eating large quantities of food, particularly grain, which increases the exogenous glucose supply. Then liver transforms glycogen to glucose, and body tissues of pregnant sheep perform the hydrolysis of fats, where the glycerin is transformed into glucose, and fatty acids oxidized due to the production of energy.

Table 30. Evaluation of Mean ± SE of Glucose (mg/dL) of Lohi sheep during different stages of pregnancy

Stages	Lohi Sheep
Non Pregnant (N.P)	39.90±0.53 NS
Early Pregnant (E.P)	45.23±0.62 NS
Mid Pregnant (M.P)	52.50±0.81 NS
Advance Pregnant (A.P)	50.10±0.74 NS
Overall Means	46.93±0.84

NS - Non-Significant

Through the use of Anlysis of variance (two way) serum cholesterol concentration has been evaluated to observe the variations in sheep groups (Table 31). Groups of Lohi sheep, various pregnant stages and groups and stages interaction had revealed statistically significant ($P \leq 0.01$) results. Mean ± SE of serum cholesterol concentration in Lohi sheep have shown an increase with the advancement of pregnancy; however, significantly different results were observed in non pregnant and early pregnant ewes; mid and advance pregnant ewes have shown a non significant increase in cholesterol concentration. Overall mean for both the groups was also significantly different (Table 32).

Table 31. Evaluation of Cholesterol (mg/dL) of Lohi sheep during different stages of pregnancy by means of using Analysis of variance

Source of Variation	Degree of Freedom	Sum of Squares	Means Squares	F-Value
Groups (G)	1	1113.778	1113.778	229.235**
Stages (S)	3	10747.484	3582.495	737.339**
G × S	3	623.634	207.878	42.785**
Error	72	349.825	4.859	
Total	39	12834.722		

**Significant - P ≤ 0.01

Table 32. Evaluation of Mean ± SE of Cholesterol (mg/dL) of Lohi sheep during different stages of pregnancy

Stages	Lohi Sheep
Non Pregnant (N.P)	30.20±0.42 [c]
Early Pregnant (E.P)	43.80±0.76 [b]
Mid Pregnant (M.P)	61.50±0.91 [a]
Advance Pregnant (A.P)	63.90±0.88 [a]
Overall Means	49.85±2.23

a-c; Table showing same alphabets do not differ significantly (P ≤ 0.01).

Serum cholesterol concentration in Lohi sheep were increased significantly from non pregnant animals to advanced stages of pregnancy, however in mid and advanced pregnant ewes this increase in cholesterol was non significant. Other research proved our results by finding significantly higher concentrations of the cholesterol in the pregnant ewes as compared to the non-pregnant ones. The reason for these increased values might be due to the enhanced lipid profile activity as indicated by increased cholesterol concentration. Resultantly, there was reduced in response of target tissue towards insulin during advanced pregnancy. These fatty acids utilization from adipose tissue was also the new source for the growth of foetus.

Significantly increased levels of cholesterol during pregnancy in sheep could be due to its role in ovarian steroidogenesis.

To evaluate variations in HDL-cholesterol level among various pregnant stages, two way Analysis of variance was applied (Table 33). Results for Lohi sheep ž groups and various pregnant stages as well as groups × stages interaction were different (P ≤ 0.01) significantly. In Lohi sheep, serum HDL-cholesterol concentration has been observed significantly (P ≤ 0.01) different, insignificant decrease in HDL-C eas observed from non-pregnant animals were shown a decrease from non pregnant stage up to advance pregnant stage progressively. The overall mean HDL-C concentration was also significantly different (Table 34).

Table 33. Evaluation of HDL-Cholesterol (HDL-C; mg/dL) of Lohi sheep during different stages of pregnancy by means of using Analysis of variance

Source of Variation	Degree of Freedom	Sum of Squares	Means Squares	F-Value
Groups (G)	1	130.050	130.050	16.139**
Stages (S)	3	6306.813	2102.271	260.882**
G × S	3	100.825	33.608	4.171**
Error	72	580.200	8.058	
Total	39	7117.888		

**Significant - P ≤ 0.01

Table 34. Evaluation of Mean ± SE of HDL-Cholesterol (HDL-C; mg/dL) of Lohi sheep during different stages of pregnancy

Stages	Lohi Sheep
Non Pregnant (N.P)	40.15±1.86 [a]
Early Pregnant (E.P)	32.75±0.86 [b]
Mid Pregnant (M.P)	27.25±0.55 [c]
Advance Pregnant (A.P)	18.60±0.64 [d]
Overall Means	29.69±1.37

a-d; Table showing same alphabets do not differ significantly (P ≤ 0.01).

Serum LDL-cholesterol concentration was determined to observe the difference between sheep groups, for this purpose two way ANOVA was used and Table 35 expressed these results. Results for Lohi sheep groups and during various pregnant stages and the groups × stages interaction have been different ($P \leq 0.01$) significantly. Mean ± SE of serum LDL-cholesterol concentration exhibited significant different results during early and mid pregnancy in ewes; an increase in LDL-C level was observed from non-pregnant to mid pregnant stage but it was decrease in advance pregnancy thus giving values similar to early pregnancy. Non pregnant and mid pregnant animals were shown significantly different values. Overall mean LDL-C concentration among various stages were revealed significantly different results not only in non pregnant animals but also throughout all the stages of pregnancy; the highest values were observed in mid pregnant animals while the lowest value was shown by non-pregnant ones. Overall mean LDL-C concentration differed significantly (Table 36).

Table 35. Evaluation of LDL-Cholesterol (LDL-C; mg/dL) of Lohi sheep during different stages of pregnancy by means of using Analysis of variance

Source of Variation	Degree of Freedom	Sum of Squares	Means Squares	F-Value
Groups (G)	1	1284.003	1284.003	276.398**
Stages (S)	3	2225.009	741.670	159.654**
G × S	3	195.609	65.203	14.036**
Error	72	334.475	4.645	
Total	39	4039.097		

**Significant - $P \leq 0.01$

Serum triglycerides concentration was observed to see the difference between sheep groups by means of using two way ANOVA and the results were presented in Table 37.

Table 36. Evaluation of Mean ± SE of LDL-Cholesterol (HDL-C; mg/dL) of Lohi sheep during different stages of pregnancy

Stages	Lohi Sheep
Non Pregnant (N.P)	27.45±0.42 [c]
Early Pregnant (E.P)	33.15±0.63 [b]
Mid Pregnant (M.P)	39.05±0.63 [a]
Advance Pregnant (A.P)	25.75±0.96 [c]
Overall Means	31.35±0.9

a-c; Table showing same alphabets do not differ significantly (P ≤ 0.01).

Lohi sheep groups, various pregnant stages and groups × stages interaction have been manifested significantly different results. Mean ± SE of serum triglycerides concentration in Lohi sheep groups observed a significant (P ≤ 0.01) increase from 1st stage of non-pregnant animals up to last stage of pregnancy (advanced pregnancy) progressively. Overall mean triglycerides concentration resulted into significantly different values (Table 38).

Table 37. Evaluation of Triglycerides (TG; mg/dL) of Lohi sheep during different stages of pregnancy by means of using Analysis of variance

Source of Variation	Degree of Freedom	Sum of Squares	Means Squares	F-Value
Groups (G)	1	6107.513	6107.513	761.127**
Stages (S)	3	34481.362	11493.787	1432.372**
G × S	3	485.363	161.788	20.162**
Error	72	577.750	8.024	
Total	39	41651.988		

**Significant - P ≤ 0.01

Table 38. Evaluation of Mean ± SE of Triglycerides (TG; mg/dL) of Lohi sheep during different stages of pregnancy

Stages	Lohi Sheep
Non Pregnant (N.P)	61.00±0.94 [d]
Early Pregnant (E.P)	87.35±0.73 [c]
Mid Pregnant (M.P)	101.55±0.73 [b]
Advance Pregnant (A.P)	123.50±0.91 [a]
Overall Means	93.35±3.65

a-d; Table showing same alphabets do not differ significantly (P ≤ 0.01).

To observe the difference between Lohi sheep for serum triiodothyronine concentration, two way ANOVA was used; Table 39 presented these results. Results for Lohi sheep groups, various pregnant stages and groups × stages interactions were differed (P ≤ 0.01) significantly. In the sheep, triiodothyronine concentration has been shown an increase from 1st stage of non-pregnant ewes throughout subsequent stages of pregnancy. Significantly different results for overall mean T_3 values were observed only during early and mid pregnant stages. Overall mean T_3 values for groups were significantly different (Table 40).

Table 39. Evaluation of Triiodothyronine (T_3; ng/mL) of Lohi sheep during different stages of pregnancy by means of using Analysis of variance

Source of Variation	Degree of Freedom	Sum of Squares	Means Squares	F-Value
Groups (G)	1	2.992	2.992	94.291**
Stages (S)	3	8.455	2.818	88.834**
G × S	3	29.121	9.707	305.960**
Error	72	2.284	0.032	
Total	39	42.852		

**Significant - P ≤ 0.01

Table 40. Evaluation of Mean ± SE of Triiodothyronine (T_3; ng/mL) of Lohi sheep during different stages of pregnancy

Stages	Lohi Sheep
Non Pregnant (N.P)	2.43±0.04 ^{c}
Early Pregnant (E.P)	2.48±0.05 ^{c}
Mid Pregnant (M.P)	3.41±0.08 ^{b}
Advance Pregnant (A.P)	4.04±0.08 ^{a}
Overall Means	3.09±0.11

a-c; Table showing same alphabets do not differ significantly (P ≤ 0.01).

Variation in the serum thyroxine concentration was evaluated by using two way ANOVA in Lohi sheep groups and Table 41 showed these results. Lohi sheep groups were not given any statistically different results. However, various pregnancy stages and groups × stages interaction exhibited significantly different (P ≤ 0.01) results. In ewes, serum thyroxine level was decreased from non-pregnant to early stage of pregnancy but increased in subsequent stages; however significantly different results were observed by advance pregnant ewes only. However, early with mid pregnant animals and advance with non pregnant animals presented similar values. Overall mean T_4 value was also significantly the same in all groups (Table 41).

Table 41. Evaluation of Thyroxinee (T_4; µg/dL) of Lohi sheep during different stages of pregnancy by means of using Analysis of variance

Source of Variation	Degree of Freedom	Sum of Squares	Means Squares	F-Value
Groups (G)	1	0.003	0.003	0.001 ^{NS}
Stages (S)	3	707.959	235.986	72.557**
G x S	3	800.109	266.703	82.001**
Error	72	234.175	3.252	
Total	39	1742.247		

**Significant - P ≤ 0.01 NS - Non-Significant

Table 42. Evaluation of Mean ± SE of Thyroxinee (T_4; µg/dL) of Lohi sheep during different stages of pregnancy

Stages	Lohi Sheep
Non Pregnant (N.P)	31.00±0.56 ^{bc}
Early Pregnant (E.P)	28.55±0.62 ^{cd}
Mid Pregnant (M.P)	31.10±0.66 ^{b}
Advance Pregnant (A.P)	39.40±0.62 ^{a}
Overall Means	32.51±0.72

a-d; Table showing same alphabets do not differ significantly (P ≤ 0.01).

By using two way analysis of variance, variations in serum cortisol concentration from sheep groups pertaining various pregnant stages was evaluated (Table 43). Lohi sheep groups at different pregnant stages and groups × stages interaction exhibited significant (P ≤ 0.01) results. Serum cortisol concentration was increased from 1st stage of non-pregnant ones up to advanced stage of pregnancy in both sheep; however, significantly different serum cortisol concentration was only observed within non pregnant ewes. Statistically different results were also observed for overall mean cortisol concentration between the groups (Table 44).

Table 43. Evaluation of Cortisol (ng/mL) of Lohi sheep during different stages of pregnancy by means of using Analysis of variance

Source of Variation	Degree of Freedom	Sum of Squares	Means Squares	F-Value
Groups (G)	1	8.385	8.385	254.202**
Stages (S)	3	29.554	9.851	298.655**
G × S	3	4.495	1.498	45.427**
Error	72	2.375	0.033	
Total	39	44.810		

**Significant - P ≤ 0.01

Table 44. Evaluation of Mean ± SE of Cortisol (ng/mL) of Lohi sheep during different stages of pregnancy

Stages	Lohi Sheep
Non Pregnant (N.P)	3.79±0.07 [d]
Early Pregnant (E.P)	4.63±0.05 [c]
Mid Pregnant (M.P)	4.74±0.05 [bc]
Advance Pregnant (A.P)	4.93±0.06 [ab]
Overall Means	4.52±0.08

a-d; Table showing same alphabets do not differ significantly (P ≤ 0.01).

5.2. Parameters during lactation

In Lohi sheep, total antioxidant status was determined by analyzing through two way Analysis of variance (Table 45). Sheep groups at various lactation stages showed significantly ($P \leq 0.01$) different results. However, a group × stages interaction did not manifest significantly different results. Total antioxidant status concentration insignificantly increased from lactation I to II while showed a non significant decrease in stage III. Although overall mean among various stages irrespective to groups were shown significantly different results; highest TAS value was observed in lactation stage II animals while the lowest was shown by lactation stage III animals. Similarly, overall mean was also significantly different.

Table 45. Evaluation of Mean ± SE of Total antioxidant status (TAS; mmol Trolox Equiv./L) of Lohi sheep during different stages of lactation

Lactation Period	Lohi Sheep
Lactation (Stage I)	2.03±0.09 [b]
Lactation (Stage II)	3.05±0.07 [a]
Lactation (Stage III)	1.76±0.04 [c]
Overall Mean	2.28±0.11

a-c; Table showing same alphabets do not differ significantly ($P \leq 0.01$).

Total oxidant status was analyzed through the use of two way analysis of variance and its variations were observed in Lohi sheep groups and Table 46 presented these results. Results for Lohi sheep groups, various lactation stages and groups and stages interaction revealed statistically significant ($P \leq 0.01$) results. In Lohi sheep, total oxidant status has differed ($P \leq 0.01$) significantly throughout all stages of lactation. Highest TOS value was observed in lactation stage-II animals while a decrease was exhibited by lactation stage I and III animals. Overall mean total oxidant status values among various lactation stages revealed statistically different ($P \leq 0.01$) results through all the stages of lactation, highest values were observed during lactation stage I whereas lactation stage III animals have shown the lowest TOS values.

Overall mean total oxidant status concentration for Lohi sheep groups displayed statistically different results.

The present findings about Lohi sheep have shown significantly results for Total Antioxidant Status. A non significant increase was observed; TAS first increased from lactation I to II stage then decreased in lactation stage III. These results were consistent with others which have also found a non significant difference of TAS in different breeds of sheep [130].

Table 46. Evaluation of Mean ± SE of Total oxidant status (TOS; μmol H_2O_2 Equiv./L) of Lohi sheep during different stages of lactation

Lactation Period	Lohi Sheep
Lactation (Stage I)	1.85±0.03 [b]
Lactation (Stage II)	2.56±0.04 [a]
Lactation (Stage III)	1.64±0.05 [c]
Overall Mean	2.02±0.08

a-c; Table showing same alphabets do not differ significantly (P ≤ 0.01).

In sheep at different lactation stages and in groups × stages interaction, significant different results (P ≤ 0.01) were obtained. Mean ± SE of serum malondialdehyde concentration was observed different (P ≤ 0.01) significantly through different lactation stages in Lohi sheep. Highest malondialdehyde value in sheep was seen at lactation stage-II and lowest during lactation stage I. Overall mean malondialdehyde values among various lactation stages have shown significant variations in lactation stage I while lactation II and III have shown significantly same results. Overall mean revealed significantly same results (Table 47).

Total Oxidant Status has shown significantly different results in ewes; highest value was observed dring lactation stage II whereas lowest by stage III animals. These results were consistent with others which have reported a significant increase in TOS concentration during 1st and 5th month of lactation in oxytocin injected buffaloes as compared to non treated animals.

There would be many factors involved in increased TOS concentration in early lactation and peak of lactation. Firstly, higher level of oxidative stress during extreme

negative energy balance (NEB) might be a sign of reduction of antioxidants precursors. Secondly, milk production is associated with oxidative stress due to the increased cellular metabolism involved and high ROMs production in low producing animals. Animals were exposed to a variety of metabolic and environmental stresses which might be the reason of rise in plasma TOS concentration.

Table 47. Evaluation of Mean ± SE of Malondialdehyde (MDA; mmol/mL) of Lohi sheep during different stages of lactation

Lactation Period	Lohi Sheep
Lactation (Stage I)	1.02±0.09 [c]
Lactation (Stage II)	2.43±0.08 [a]
Lactation (Stage III)	2.08±0.09 [b]
Overall Mean	1.84±0.12

a-c; Table showing same alphabets do not differ significantly (P ≤ 0.01).

Highest value of MDA was observed during mid lactation in Lohi sheep. These results were in line with the findings of other research confirmed that there was clearly an increase in the MDA level from 1 day after lambing i.e. early lactation earlier than the 8th week of lactation i.e. mid lactation observed in sheep.

The likely cause of this rise in MDA concentration could be due to overproduction of free radicals and thus induction of lipid peroxidation. The other reason for steady increase of MDA concentration with the advancement of lactation was due to hypothyroidism. Decreased level of T3 and T4 with the advancement of lactation in our findings also confirmed these.

In Lohi sheep serum superoxide dismutase concentration was shown significantly (P ≤ 0.01) different results during various stages of lactation; highest superoxide dismutase value was observed in lactation stage-II followed a decrease by lactation stage-III and stage I animals. Irrespective to groups, overall mean SOD values for various lactation stages were found significant through all the lactation stages, followed similar pattern of highest value by lactation stage II and lowest among stage I animals (Table 48).

Table 48. Evaluation of Mean ± SE of Superoxide dismutase (SOD; µ/mL) of Lohi sheep during different stages of lactation

Lactation Period	Lohi Sheep
Lactation (Stage I)	1.58±0.06 [c]
Lactation (Stage II)	2.57±0.04 [a]
Lactation (Stage III)	2.07±0.08 [b]
Overall Mean	2.07±0.08

a-c; Table showing same alphabets do not differ significantly (P ≤ 0.01).

In Lohi sheep, catalase concentration has been manifested significantly different results in lactation stage-I; significantly same values were obtained in stage II and III. Overall mean serum catalase values among various lactation stages irrespective to sheep groups have shown significantly (P ≤ 0.01) different results throughout all the stages of lactation; highest values were observed by lactating stage II animals following a significant decrease by stage III and I animals (Table 49).

Table 49. Evaluation of Mean ± SE of Catalase (CAT; KU/L) of Lohi sheep during different stages of lactation

Lactation Period	Lohi Sheep
Lactation (Stage I)	1.23±0.05 [b]
Lactation (Stage II)	2.48±0.07 [a]
Lactation (Stage III)	2.46±0.05 [a]
Overall Mean	2.06±0.11

a-b; Table showing same alphabets do not differ significantly (P ≤ 0.01).

In Lohi sheep, vitamin E concentration decreased insignificantly from lactation stage I to II and than an increase was observed during lactation stage III which was also non significant. Irrespective to sheep groups, overall mean vitamin E concentration among various stages was found significant throughout all the stages of lactation; lowest vitamin E values were observed by lactation stage-II animals while highest by lactation stage-III animals (Table 50).

Table 50. Evaluation of Mean ± SE of Vitamin E (Vit. E; µmol/L) of Lohi sheep during different stages of lactation

Lactation Period	Lohi Sheep
Lactation (Stage I)	1.40±0.01 NS
Lactation (Stage II)	1.29±0.02 NS
Lactation (Stage III)	1.51±0.01 NS
Overall Mean	1.40±0.02

NS - Non-Significant

A non significant increase was observed from stage-I to lactation stage II that was, however, decreased insignificantly during lactation stage-III in Lohi. Overall mean serum vitamin C concentration among different stages of lactation irrespective to groups was found significantly different throughout all the lactation stages; the highest vit. C value was observed at lactation stage II and lowest was found during lactation stage III (Table 51).

Table 51. Evaluation of Mean ± SE of Vitamin C (Vit. C; µmol/L) of Lohi sheep during different stages of lactation

Lactation Period	Lohi Sheep
Lactation (Stage I)	4.03±0.04 NS
Lactation (Stage II)	4.91±0.08 NS
Lactation (Stage III)	3.07±0.08 NS
Overall Mean	4.00±0.14

NS - Non-Significant

Serum PON I concentration has not shown significantly different values through various stages of lactation in sheep groups; highest value was shown at lactation stage II than decreased non significantly in stage III and I of lactation. Overall mean paraoxonase values among various lactation stages were found significantly different throughout all stages of lactation; the highest PON-I value was observed during lactation stage II and lowest was during lactation stage I (Table 52).

Table 52. Evaluation of Mean ± SE of Paraoxonase (PON-I; U/min/mL) of Lohi sheep during different stages of lactation

Lactation Period	Lohi Sheep
Lactation (Stage I)	148.50±9.60 NS
Lactation (Stage II)	174.50±1.04 NS
Lactation (Stage III)	165.50±1.23 NS
Overall Mean	162.83±3.72

NS - Non-Significant

When arylesterase concentration was studied during various stages, insignificant decreased values were noted from early (I) to mid stage of lactation (II) than an increase was seen during late stage (III). Overall mean arylesterase values among various stages were found significantly ($P \leq 0.01$) different through all the lactation stages, highest values were found in lactating stage I animals and lowest by the animals at lactation stage II (Table 53).

Table 53. Evaluation of Mean ± SE of Arylesterase (Ary; KU/min/L) of Lohi sheep during different stages of lactation

Lactation Period	Lohi Sheep
Lactation (Stage I)	62.60±0.69 a
Lactation (Stage II)	52.45±1.03 c
Lactation (Stage III)	57.00±1.37 b
Overall Mean	57.35±0.97

a-c; Table showing same alphabets do not differ significantly ($P \leq 0.01$).

Serum ceruloplasmin concentration in Lohi sheep has shown significantly different results only during lactation stage-II; lactation stage I and III were given significantly the same values. Overall mean ceruloplasmin values through various stages of lactation irrespective to groups were significantly different throughout all the lactation stages; highest Cp values were found during lactation stage II followed a significant decrease in lactation stage III and stage I animals (Table 54).

Table 54. Evaluation of Mean ± SE of Ceruloplasmin (Cp; U/L) of Lohi sheep during different stages of lactation

Lactation Period	Lohi Sheep
Lactation (Stage I)	132.05±1.47 [b]
Lactation (Stage II)	141.10±0.81 [a]
Lactation (Stage III)	128.40±1.01 [b]
Overall Mean	133.85±1.17

a-b; Table showing same alphabets do not differ significantly (P ≤ 0.01).

There was observed non significant decrease in homocysteine concentration from lactation stage I to III in sheep. Overall mean homocysteine values among various stages were found significantly different throughout all the lactation stages; a significant decrease in Hcy was observed from lactation stage I to stage III animals (Table 55).

Table 55. Evaluation of Mean ± SE of Homocysteine (Hcy; µmol/L) of Lohi sheep during different stages of lactation

Lactation Period	Lohi Sheep
Lactation (Stage I)	64.20±0.79 [a]
Lactation (Stage II)	48.10±0.69 [b]
Lactation (Stage III)	42.20±0.59 [c]
Overall Mean	51.50±1.77

a-c; Table showing same alphabets do not differ significantly (P ≤ 0.01).

In Lohi sheep groups, mean ± SE of serum aspartate aminotransferase concentration was not manifested significantly different results; although the highest AST value was showed by lactation stage I animals than a non significant decrease was observed among various stages of lactation in a sequence. Overall mean aspartate aminotransferase values among various lactation stages showed significantly different results throughout all the stages; a significant decrease was observed from lactation stage I to lactation stage III (Table 56).

Table 56. Evaluation of Mean ± SE of Aspartate aminotransferase (AST; U/L) of Lohi sheep during different stages of lactation

Lactation Period	Lohi Sheep
Lactation (Stage I)	56.85±0.84 NS
Lactation (Stage II)	46.00±0.56 NS
Lactation (Stage III)	43.45±0.30 NS
Overall Mean	48.77±1.13

NS - Non-Significant

Mean ± SE of serum alanine aminotransferase concentration in sheep was not differ significantly during all the stages of lactation; however the value of ALT was observed significantly increased from stage-I of lactation to stage-III in a sequence. A significant increased ALT concentration was observed from lactation stage I to stage III (Table 57).

Table 57. Evaluation of Mean ± SE of Alanine aminotransferase (ALT; U/L) of Lohi sheep during different stages of lactation

Lactation Period	Lohi Sheep
Lactation (Stage I)	8.37±0.14 c
Lactation (Stage II)	15.83±0.38 b
Lactation (Stage III)	18.43±0.25 a
Overall Mean	14.21±0.81

a-c; Table showing same alphabets do not differ significantly (P ≤ 0.01).

The highest GGT level was observed in lactation stage-I animals than a significant decrease has been noted from mid (II) to late (III) lactation respectively in both ewes and does. Moreover, Lohi sheep did show much higher gamma-glutamyl transferase concentration during all the lactation stages. Overall mean GGT values among various stages irrespective to groups exhibited significantly different results through all the lactation stages thus followed similar sequence of significant decrease from lactation stage I to stage III (Table 58).

Table 58. Evaluation of Mean ± SE of Gamma-glutamyl transferase (GGT; U/L) of Lohi sheep during different stages of lactation

Lactation Period	Lohi Sheep
Lactation (Stage I)	24.50±0.50 [a]
Lactation (Stage II)	16.90±0.53 [b]
Lactation (Stage III)	15.05±0.37 [c]
Overall Mean	18.82±0.8

a-c; Table showing same alphabets do not differ significantly (P ≤ 0.01).

Glucose concentration in sheep throughout all the lactation stages has not been manifested different results significantly. A non significant increase in glucose concentration was observed from stage-I to stage-II of lactation that was however decreased during lactation stage-III (Table 59).

Table 59. Evaluation of Mean ± SE of Glucose (mg/dL) of Lohi sheep during different stages of lactation

Lactation Period	Lohi Sheep
Lactation (Stage I)	36.15±0.43 [NS]
Lactation (Stage II)	41.80±0.53 [NS]
Lactation (Stage III)	32.55±1.00 [NS]
Overall Mean	36.83±0.81

NS - Non-Significant

In Lohi sheep, serum cholesterol concentration has shown an increase from stage-I of lactation to stage-II that decreased in stage-III; lactation stage I and III showed significantly the same cholesterol concentration. Overall mean cholesterol values among various stages have showed different (P ≤ 0.01) results throughout all the lactation stages in both the groups thus followed similar pattern of highest values at lactation-II and lowest at III stage (Table 60).

Table 60. Evaluation of Mean ± SE of Cholesterol (mg/dL) of Lohi sheep during different stages of lactation

Lactation Period	Lohi Sheep
Lactation (Stage I)	41.80±0.61 [b]
Lactation (Stage II)	52.00±0.94 [a]
Lactation (Stage III)	40.10±0.90 [cb]
Overall Mean	44.63±1.08

a-c; Table showing same alphabets do not differ significantly (P ≤ 0.01).

Serum HDL-cholesterol concentration has been found significantly similar in sheep during various stages of lactation; non-significantly highest and lowest values for HDL-C concentration were observed by lactation stage II and lactation stage I animals respectively. Overall mean HDL-C concentration between groups has not revealed significantly different results (Table 61).

Table 62. Evaluation of Mean ± SE of HDL-Cholesterol (HDL-C; mg/dL) of Lohi sheep during different stages of lactation

Lactation Period	Lohi Sheep
Lactation (Stage I)	23.15±0.71 [NS]
Lactation (Stage II)	35.40±0.97 [NS]
Lactation (Stage III)	27.55±0.49 [NS]
Overall Mean	28.70±1.03

NS - Non-Significant

Mean ± SE of serum LDL-C concentration in Lohi sheep exhibited statistically different results only during lactation stage-III; lactation stage I and II showed significantly same values. Overall mean LDL-C concentration among various lactation stages showed significantly different (P ≤ 0.01) results during lactation stages (Table 63).

Table 63. Evaluation of Mean ± SE of LDL-Cholesterol (LDL-C; mg/dL) of Lohi sheep during different stages of lactation

Lactation Period	Lohi Sheep
Lactation (Stage I)	22.45±0.82 [b]
Lactation (Stage II)	20.00±0.88 [b]
Lactation (Stage III)	27.00±0.58 [a]
Overall Mean	23.15±0.69

a-b; Table showing same alphabets do not differ significantly (P ≤ 0.01).

Serum triglycerides level was decreased significantly from lactation stage-I to stage-III in Lohi sheep. Overall mean TG values among various stages, serum triglycerides has shown statistically significant (P ≤ 0.01) values throughout all the stages of lactation; triglycerides concentration was significantly increased from stage-I of lactation to stage-III in a sequence (Table 64).

Table 64. Evaluation of Mean ± SE of Triglycerides (TG; mg/dL) of Lohi sheep during different stages of lactation

Lactation Period	Lohi Sheep
Lactation (Stage I)	107.00±1.78 [a]
Lactation (Stage II)	78.85±0.94 [b]
Lactation (Stage III)	58.70±0.93 [c]
Overall Mean	81.52±3.75

a-c; Table showing same alphabets do not differ significantly (P ≤ 0.01).

Serum triiodothyronine level has observed different (P ≤ 0.01) significantly only during stage-I of in Lohi sheep; whereas lactation stage-II and III have shown significantly the same values. Overall mean triiodothyronine concentration among different lactation stages were found significantly (P ≤ 0.01) different throughout all the stages of lactation, highest value was found during lactation stage I and lowest by lactation stage II animals (Table 65).

Table 65. Evaluation of Mean ± SE of Triiodothyronine (T_3; ng/mL) of Lohi sheep during different stages of lactation

Lactation Period	Lohi Sheep
Lactation (Stage I)	1.68±0.05 [a]
Lactation (Stage II)	1.37±0.01 [b]
Lactation (Stage III)	1.33±0.01 [b]
Overall Mean	1.46±0.03

a-b; Table showing same alphabets do not differ significantly (P ≤ 0.01).

Serum thyroxine level has significantly been different in lactation-I in Lohi sheep. Overall mean thyroxine values among various stages of lactation were observed significant throughout all the stages of lactation; a significant decrease was observed from lactation stage I to stage III in a sequence (Table 66).

Table 66. Evaluation of Mean ± SE of Thyroxine (T_4; µg/dL) of Lohi sheep during different stages of lactation

Lactation Period	Lohi Sheep
Lactation (Stage I)	32.75±0.55 [a]
Lactation (Stage II)	26.55±0.44 [b]
Lactation (Stage III)	27.40±0.28 [b]
Overall Mean	28.90±0.57

a-b; Table showing same alphabets do not differ significantly (P ≤ 0.01).

Serum cortisol level during various lactation stages has also exhibited significantly same results. Insignificant decrease in cortisol concentration has been noted from stage-I of lactation to stage-III. Overall mean serum cortisol concentration among various lactation stages was observed significantly different throughout all lactation stages; a significant decrease in cortisol concentration was found from lactation stage I to stage-III (Table 67).

Table 67. Evaluation of Mean ± SE of Cortisol (ng/mL) of Lohi sheep during different stages of lactation

Lactation Period	Lohi Sheep
Lactation (Stage I)	3.53±0.09 NS
Lactation (Stage II)	2.58±0.08 NS
Lactation (Stage III)	2.02±0.06 NS
Overall Mean	2.71±0.12

NS - Non-Significant

In recent studies, serum cortisol concentration has shown non significant results throughout various stages of lactation in sheep. However, non significant decrease in cortisol concentration was observed through various stages of lactation. Others observed the same results [131] of non significant decrease in cortisol concentration during different lactation periods. Similarly, other research resulted in decreased cortisol concentration through various stages of lactation [132]. It was reported [133] significantly the same results in control group as compared to vitamin C supplemented sheep which showed significantly decreased level of cortisol durin lactation.

6. CONCLUSIONS

Prooxidant or antioxidant statuses along with biochemical parameters were considered useful tools for evaluation of oxidative stress during physiologically stress condition of sheep gestation. Additionally, these tools might be useful for improved management strategies during farm conditions in ewes and even goats. Animals were facing oxidative stress as indicated through increased prooxidants; MDA, TOS and decrease in antioxidant capacity; TAS, SOD, CAT, PON-I and HCy with the progression of pregnancy. Likewise, striking changes in various enzymes, hormones and energy metabolites were the requirement of physiological adaptation during stress period.

As the lactation is the most energy demanding period in the life of organism as the production of reactive oxygen species were enhanced and oxidative stress is positively correlated with the production of the animal. The markers of oxidative status and metabolic profile along with enzymes and hormones in lactating ewes undergoes maximum strain and animals have been experienced oxidative stress in early lactation and at peak of lactation time than it gradually diminished.

The use of oxidants and antioxidants markers along with blood metabolic profile is one of the recommended procedures for monitoring health status during this critical period and to evade reduced productive performance and economic losses.

REFERENCES

1. Boukhenouna, S.; Wilson, M.A.; Bahmed, K.; Kosmider, B. Reactive Oxygen Species in Chronic Obstructive Pulmonary Disease. *Oxidative Medicine and Cellular Longevity* **2018**, *2018*, 1–9, doi:10.1155/2018/5730395.

2. Taverne, Y.J.; Merkus, D.; Bogers, A.J.; Halliwell, B.; Duncker, D.J.; Lyons, T.W. Reactive Oxygen Species: Radical Factors in the Evolution of Animal Life: A Molecular Timescale from Earth's Earliest History to the Rise of Complex Life. *BioEssays* **2018**, *40*, 1700158, doi:10.1002/bies.201700158.

3. Kostadinović, L.M.; Popović, S.J.; Puvača, N.M.; Čabarkapa, I.S.; Kormanjoš, Š.M.; Lević, J.D. Influence of Artemisia Absinthium Essential Oil on Antioxidative System of Broilers Experimentally Infected with Eimeria Oocysts. *Veterinarski arhiv* **2016**, *86*, 253–264.

4. Puvača, N.; Kostadinović, Lj.; Popović, S.; Lević, J.; Ljubojević, D.; Tufarelli, V.; Jovanović, R.; Tasić, T.; Ikonić, P.; Lukač, D. Proximate Composition, Cholesterol Concentration and Lipid Oxidation of Meat from Chickens Fed Dietary Spice Addition (Allium Sativum, Piper Nigrum, Capsicum Annuum). *Anim. Prod. Sci.* **2016**, *56*, 1920–1927, doi:10.1071/AN15115.

5. Gessner, D.K.; Ringseis, R.; Eder, K. Potential of Plant Polyphenols to Combat Oxidative Stress and Inflammatory Processes in Farm Animals. *J Anim Physiol Anim Nutr* **2017**, *101*, 605–628, doi:10.1111/jpn.12579.

6. Kryl'skii, E.D.; Popova, T.N.; Safonova, O.A.; Stolyarova, A.O.; Razuvaev, G.A.; de Carvalho, M.A.P. Transcriptional Regulation of Antioxidant Enzymes Activity and Modulation of Oxidative Stress by Melatonin in Rats Under Cerebral Ischemia / Reperfusion Conditions. *Neuroscience* **2019**, *406*, 653–666, doi:10.1016/j.neuroscience.2019.01.046.

7. Barati, E.; Nikzad, H.; Karimian, M. Oxidative Stress and Male Infertility: Current Knowledge of Pathophysiology and Role of Antioxidant Therapy in Disease Management. *Cell. Mol. Life Sci.* **2020**, *77*, 93–113, doi:10.1007/s00018-019-03253-8.

8. Burgos-Morón; Abad-Jiménez; Marañón; Iannantuoni; Escribano-López; López-Domènech; Salom; Jover; Mora; Roldan; et al. Relationship Between Oxidative Stress, ER Stress, and Inflammation in Type 2 Diabetes: The Battle Continues. *JCM* **2019**, *8*, 1385, doi:10.3390/jcm8091385.

9. Beit-Hallahmi, B. Developing the Prison Fantasy Questionnaire (PFQ). *J Clin Psychol* **1972**, *28*, 551–554, doi:10.1002/1097-4679(197210)28:4<551::aid-jclp2270280433>3.0.co;2-o.

10. Sinha, T.; Naash, M.I.; Al-Ubaidi, M.R. Flavins Act as a Critical Liaison Between Metabolic Homeostasis and Oxidative Stress in the Retina. *Front. Cell Dev. Biol.* **2020**, *8*, 861, doi:10.3389/fcell.2020.00861.

11. Tashla, T.; Puvača, N.; Pelić, D.L.; Prodanović, R.; Bošković, J.; Ivanišević, D.; Jahić, M.; Mahmoud, O.; Giannenas, I.; Lević, J. Dietary Medicinal Plants Enhance the Chemical Composition and Quality of Broiler Chicken Meat. *J Hellenic Vet Med Soc* **2019**, *70*, 1823–1832, doi:10.12681/jhvms.22229.

12. Cao, C.; Zhang, H.; Wang, K.; Li, X. Selenium-Rich Yeast Mitigates Aluminum-Mediated Testicular Toxicity by Blocking Oxidative Stress, Inhibiting NO Production, and Disturbing Ionic Homeostasis. *Biol Trace Elem Res* **2020**, *195*, 170–177, doi:10.1007/s12011-019-01820-5.

13. Cecchini, S.; Fazio, F.; Bazzano, M.; Caputo, A.R.; Giannetto, C.; Piccione, G. Redox Status and Oxidative Stress during Late Pregnancy and Postpartum Period in Mares. *Livestock Science* **2019**, *230*, 103821, doi:10.1016/j.livsci.2019.103821.

14. Alharthi, A.; Zhou, Z.; Lopreiato, V.; Trevisi, E.; Loor, J.J. Body Condition Score Prior to Parturition Is Associated with Plasma and Adipose Tissue Biomarkers of Lipid Metabolism and Inflammation in Holstein Cows. *J Animal Sci Biotechnol* **2018**, *9*, 12, doi:10.1186/s40104-017-0221-1.

15. Masters, D.G. Practical Implications of Mineral and Vitamin Imbalance in Grazing Sheep. *Anim. Prod. Sci.* **2018**, *58*, 1438, doi:10.1071/AN17761.

16. Lewis, N.; Hinrichs, K.; Leese, H.J.; McG. Argo, C.; Brison, D.R.; Sturmey, R. Energy Metabolism of the Equine Cumulus Oocyte Complex during in Vitro Maturation. *Sci Rep* **2020**, *10*, 3493, doi:10.1038/s41598-020-60624-z.

17. Limesand, S.W.; Camacho, L.E.; Kelly, A.C.; Antolic, A.T. Impact of Thermal Stress on Placental Function and Fetal Physiology. *Anim Reprod* **2018**, *15*, 886–898, doi:10.21451/1984-3143-AR2018-0056.

18. Anadón, A.; Ares, I.; Martínez-Larrañaga, M.R.; Martínez, M.A. Nutraceuticals Used as Antibacterial Alternatives in Animal Health and Disease. In *Nutraceuticals in Veterinary Medicine*; Gupta, R.C., Srivastava, A., Lall, R., Eds.; Springer International Publishing: Cham, 2019; pp. 315–343 ISBN 978-3-030-04623-1.

19. Kalyesubula, M.; Rosov, A.; Alon, T.; Moallem, U.; Dvir, H. Intravenous Infusions of Glycerol Versus Propylene Glycol for the Regulation of Negative Energy Balance in Sheep: A Randomized Trial. *Animals* **2019**, *9*, 731, doi:10.3390/ani9100731.

20. Zhang, H.; Zhao, F.; Nie, H.; Ma, T.; Wang, Z.; Wang, F.; Loor, J.J. Dietary N-Carbamylglutamate and Rumen-Protected L-Arginine Supplementation during Intrauterine Growth Restriction in Undernourished Ewes Improve Fetal Thymus Development and Immune Function. *Reprod. Fertil. Dev.* **2018**, *30*, 1522, doi:10.1071/RD18047.

21. Mavangira, V.; Sordillo, L.M. Role of Lipid Mediators in the Regulation of Oxidative Stress and Inflammatory Responses in Dairy Cattle. *Research in Veterinary Science* **2018**, *116*, 4–14, doi:10.1016/j.rvsc.2017.08.002.

22. Staerck, C.; Gastebois, A.; Vandeputte, P.; Calenda, A.; Larcher, G.; Gillmann, L.; Papon, N.; Bouchara, J.-P.; Fleury, M.J.J. Microbial Antioxidant Defense Enzymes. *Microbial Pathogenesis* **2017**, *110*, 56–65, doi:10.1016/j.micpath.2017.06.015.

23. Chekima, B.; Chekima, K.; Chekima, K. Understanding Factors Underlying Actual Consumption of Organic Food: The Moderating Effect of Future Orientation. *Food Quality and Preference* **2019**, *74*, 49–58, doi:10.1016/j.foodqual.2018.12.010.

24. Faverzani, J.L.; Hammerschmidt, T.G.; Sitta, A.; Deon, M.; Wajner, M.; Vargas, C.R. Oxidative Stress in Homocystinuria Due to Cystathionine SS-Synthase Deficiency: Findings in Patients and in Animal Models. *Cell Mol Neurobiol* **2017**, *37*, 1477–1485, doi:10.1007/s10571-017-0478-0.

25. Singh, N.; NaveenKumar, S.K.; Geethika, M.; Mugesh, G. A Cerium Vanadate Nanozyme with Specific Superoxide Dismutase Activity Regulates Mitochondrial Function and ATP Synthesis in Neuronal Cells. *Angew. Chem. Int. Ed.* **2020**, anie.202011711, doi:10.1002/anie.202011711.

26. Górnicka, M.; Ciecierska, A.; Hamulka, J.; Drywień, M.E.; Frackiewicz, J.; Górnicki, K.; Wawrzyniak, A. α -Tocopherol Protects the Heart, Muscles, and Testes from Lipid Peroxidation in Growing Male Rats Subjected to Physical Efforts. *Oxidative Medicine and Cellular Longevity* **2019**, *2019*, 1–13, doi:10.1155/2019/8431057.

27. Klichkhanov, N.K.; Ismailova, Zh.G.; Astaeva, M.D.; Chalabov, Sh.I. Effects of Vitamins C and E on Free Radical Processes in the Blood of Rats in Acute Moderate Hypothermia. *Biol Bull Russ Acad Sci* **2019**, *46*, 536–543, doi:10.1134/S1062359019060086.

28. Gusev, E.Yu.; Zotova, N.V. Cellular Stress and General Pathological Processes. *CPD* **2019**, *25*, 251–297, doi:10.2174/1381612825666190319114641.

29. Khalil, A.; Fulop, T.; Berrougui, H. Role of Paraoxonase1 in the Regulation of High-Density Lipoprotein Functionality and in Cardiovascular Protection. *Antioxidants & Redox Signaling* **2021**, *34*, 191–200, doi:10.1089/ars.2019.7998.

30. Meneses; Silvestre; Sousa-Lima; Macedo Paraoxonase-1 as a Regulator of Glucose and Lipid Homeostasis: Impact on the Onset and Progression of Metabolic Disorders. *IJMS* **2019**, *20*, 4049, doi:10.3390/ijms20164049.

31. Choromańska, B.; Myśliwiec, P.; Łuba, M.; Wojskowicz, P.; Dadan, J.; Myśliwiec, H.; Choromańska, K.; Zalewska, A.; Maciejczyk, M. A Longitudinal Study of the Antioxidant Barrier and Oxidative Stress in Morbidly Obese Patients after Bariatric Surgery. Does the Metabolic Syndrome Affect the Redox Homeostasis of Obese People? *JCM* **2020**, *9*, 976, doi:10.3390/jcm9040976.

32. Karasahin, T.; Alkan, H.; Satilmis, F.; Dursun, S.; Ozturk, C.; Bulut, G.; Aksoy, N.H.; Tekindal, M.A.; Caglayan, T.; Yesilkaya, O.F.; et al. Relationship between Total Antioxidant/Oxidant Status, and Oxidative Stress Index and Superovulation Response in Donor Cows. *Livestock Science* **2020**, 104340, doi:10.1016/j.livsci.2020.104340.

33. El-Sayed, A. Evaluation of Hematological and Biochemical Changes in Dromedary Camel during the Different Stages of Lactation. *Mans Vet Med J* **2020**, *21*, 121–124, doi:10.35943/mvmj.2020.21.320.

34. ten Berge, H.F.M.; Hijbeek, R.; van Loon, M.P.; Rurinda, J.; Tesfaye, K.; Zingore, S.; Craufurd, P.; van Heerwaarden, J.; Brentrup, F.; Schröder, J.J.; et al. Maize Crop Nutrient Input Requirements for Food Security in Sub-Saharan Africa. *Global Food Security* **2019**, *23*, 9–21, doi:10.1016/j.gfs.2019.02.001.

35. Takaeko, Y.; Kajikawa, M.; Kishimoto, S.; Yamaji, T.; Harada, T.; Han, Y.; Kihara, Y.; Hida, E.; Chayama, K.; Goto, C.; et al. Low Levels of Low-Density Lipoprotein Cholesterol and Endothelial Function in Subjects without Lipid-Lowering Therapy. *JCM* **2020**, *9*, 3796, doi:10.3390/jcm9123796.

36. Ibtisham, F.; Nawab, A.; Li, G.; Xiao, M.; An, L.; Naseer, G. Effect of Nutrition on Reproductive Efficiency of Dairy Animals. *Medycyna Weterynaryjna* **2018**, *74*, 6025–2018, doi:10.21521/mw.6025.

37. Ramírez, S.; Gómez-Valadés, A.G.; Schneeberger, M.; Varela, L.; Haddad-Tóvolli, R.; Altirriba, J.; Noguera, E.; Drougard, A.; Flores-Martínez, Á.; Imbernón, M.; et al. Mitochondrial Dynamics Mediated by Mitofusin 1 Is Required for POMC Neuron Glucose-Sensing and Insulin Release Control. *Cell Metabolism* **2017**, *25*, 1390-1399.e6, doi:10.1016/j.cmet.2017.05.010.

38. Fan, Y.; Han, Z.; Lu, X.; Zhang, H.; Arbab, A.A.I.; Loor, J.J.; Yang, Y.; Yang, Z. Identification of Milk Fat Metabolism-Related Pathways of the Bovine Mammary Gland during Mid and Late Lactation and Functional Verification of the ACSL4 Gene. *Genes* **2020**, *11*, 1357, doi:10.3390/genes11111357.

39. Lopez-Vicchi, F.; Winne, C.D.; Brie, B.; Sorianello, E.; Ladyman, S.R.; Becu-Villalobos, D. Metabolic Functions of Prolactin: Physiological and Pathological Aspects. *Journal of Neuroendocrinology* **2020**, *32*, e12888, doi:https://doi.org/10.1111/jne.12888.

40. Wang, Y.; Wu, T.; Zang, X.; Liu, X.; Xu, W.; Lai, P.; Wang, Y.; Teng, F.; Qiu, Q.; Geng, H.; et al. Relationship Between Serum Gamma-Glutamyl Transferase Level and Impaired Fasting Glucose Among Chinese Community-Dwelling Adults: A Follow-Up Observation of 6 Years. *Metabolic Syndrome and Related Disorders* **2020**, doi:10.1089/met.2020.0032.

41. Kafilzadeh, F.; Payandeh, S.; Gómez-Cortés, P.; Ghadimi, D.; Schiavone, A.; Marín, A.L.M. Effects of Probiotic Supplementation on Milk Production, Blood Metabolite Profile and Enzyme Activities of Ewes during Lactation. *Italian Journal of Animal Science* **2019**, *18*, 134–139, doi:10.1080/1828051X.2018.1496040.

42. Koohpeyma, H.; Goudarzi, I.; Elahdadi Salmani, M.; Lashkarbolouki, T.; Shabani, M. Folic Acid Protects Rat Cerebellum Against Oxidative Damage Caused by Homocysteine: The Expression of Bcl-2, Bax, and Caspase-3 Apoptotic Genes. *Neurotox Res* **2020**, *37*, 564–577, doi:10.1007/s12640-019-00119-6.

43. Veena, M.P.; Swamy, M.N.; Ramesh, P.T.; Rao, S.; Shridhar, N.B.; Kalmath, G.P.; Swetha, H.S. Dynamics of Thyroid Hormones in Growth and Development of South Indian Sheep (Bannur). **2018**, 8.

44. Putman, A.K.; Brown, J.L.; Gandy, J.C.; Wisnieski, L.; Sordillo, L.M. Changes in Biomarkers of Nutrient Metabolism, Inflammation, and Oxidative Stress in Dairy Cows during the Transition into the Early Dry Period. *Journal of Dairy Science* **2018**, *101*, 9350–9359, doi:10.3168/jds.2018-14591.

45. Rout, P.K.; Behera, B.K. Goat and Sheep Farming. In *Sustainability in Ruminant Livestock*; Springer Singapore: Singapore, 2021; pp. 33–76 ISBN 978-981-334-342-9.

46. Gowane, G.R.; Gadekar, Y.P.; Prakash, V.; Kadam, V.; Chopra, A.; Prince, L.L.L. Climate Change Impact on Sheep Production: Growth, Milk, Wool, and Meat. In *Sheep Production Adapting to Climate Change*; Sejian, V., Bhatta, R., Gaughan, J., Malik, P.K., Naqvi, S.M.K., Lal, R., Eds.; Springer Singapore: Singapore, 2017; pp. 31–69 ISBN 978-981-10-4713-8.

47. El-Ratel, I.T.; Abdel-Khalek, A.E.; Gabr, S.A.; Hammad, M.E.; El-Morsy, H.I. Influence of Allicin Administration on Reproductive Efficiency, Immunity and Lipid

Peroxidation of Rabbit Does under High Ambient Temperature. *J Anim Physiol Anim Nutr* **2020**, *104*, 539–548, doi:10.1111/jpn.13316.

48. Saleem, A.H.; Javed, K.; Babar, M.E.; Hussain, T.; Ali, A.; Ali, A.; Ahmad, N.; Farooq, M.Z.; Dawood, M. Association of Leptin Gene Polymorphism with Growth Rate in Lohi Sheep. *PJZ* **2018**, *50*, doi:10.17582/journal.pjz/2018.50.3.1029.1033.

49. Era's Lucknow Medical College & Hospital, Sarfarazganj Lucknow, U.P., India-226003; Kumar, V.; Salam, A. A REVIEW ON REACTIVE OXYGEN AND NITROGEN SPECIES. *EJMR* **2018**, *5*, 59–66, doi:10.24041/ejmr2018.63.

50. Olivo, G.; Cussó, O.; Borrell, M.; Costas, M. Oxidation of Alkane and Alkene Moieties with Biologically Inspired Nonheme Iron Catalysts and Hydrogen Peroxide: From Free Radicals to Stereoselective Transformations. *J Biol Inorg Chem* **2017**, *22*, 425–452, doi:10.1007/s00775-016-1434-z.

51. Simões, E.F.C.; Almeida, A.S.; Duarte, A.C.; Duarte, R.M.B.O. Assessing Reactive Oxygen and Nitrogen Species in Atmospheric and Aquatic Environments: Analytical Challenges and Opportunities. *TrAC Trends in Analytical Chemistry* **2021**, *135*, 116149, doi:10.1016/j.trac.2020.116149.

52. Sies, H.; Jones, D.P. Reactive Oxygen Species (ROS) as Pleiotropic Physiological Signalling Agents. *Nat Rev Mol Cell Biol* **2020**, *21*, 363–383, doi:10.1038/s41580-020-0230-3.

53. Kamm, A.; Przychodzen, P.; Kuban-Jankowska, A.; Jacewicz, D.; Dabrowska, A.M.; Nussberger, S.; Wozniak, M.; Gorska-Ponikowska, M. Nitric Oxide and Its Derivatives in the Cancer Battlefield. *Nitric Oxide* **2019**, *93*, 102–114, doi:10.1016/j.niox.2019.09.005.

54. Gebhart, V.; Reiß, K.; Kollau, A.; Mayer, B.; Gorren, A.C.F. Site and Mechanism of Uncoupling of Nitric-Oxide Synthase: Uncoupling by Monomerization and Other Misconceptions. *Nitric Oxide* **2019**, *89*, 14–21, doi:10.1016/j.niox.2019.04.007.

55. Popović, S.; Puvača, N.; Kostadinović, L.; Džinić, N.; Bošnjak, J.; Vasiljević, M.; Djuragic, O. Effects of Dietary Essential Oils on Productive Performance, Blood Lipid Profile, Enzyme Activity and Immunological Response of Broiler Chickens. *Europ.Poult.Sci* **2016**, *80*, doi:DOI: 10.1399/eps.2016.146.

56. Gau, E.; Flecken, F.; Ksiazkiewicz, A.N.; Pich, A. Enzymatic Synthesis of Temperature-Responsive Poly(*N* -Vinylcaprolactam) Microgels with Glucose Oxidase. *Green Chem.* **2018**, *20*, 431–439, doi:10.1039/C7GC03111D.

57. Yaribeygi, H.; Atkin, S.L.; Sahebkar, A. A Review of the Molecular Mechanisms of Hyperglycemia-Induced Free Radical Generation Leading to Oxidative Stress: YARIBEYGI ET AL. *J Cell Physiol* **2019**, *234*, 1300–1312, doi:10.1002/jcp.27164.

58. Perry, S.C.; Pangotra, D.; Vieira, L.; Csepei, L.-I.; Sieber, V.; Wang, L.; Ponce de León, C.; Walsh, F.C. Electrochemical Synthesis of Hydrogen Peroxide from Water and Oxygen. *Nat Rev Chem* **2019**, *3*, 442–458, doi:10.1038/s41570-019-0110-6.

59. Lees, J.G.; Gardner, D.K.; Harvey, A.J. Pluripotent Stem Cell Metabolism and Mitochondria: Beyond ATP. *Stem Cells International* **2017**, *2017*, 1–17, doi:10.1155/2017/2874283.

60. Radi, R. Oxygen Radicals, Nitric Oxide, and Peroxynitrite: Redox Pathways in Molecular Medicine. *Proc Natl Acad Sci USA* **2018**, *115*, 5839–5848, doi:10.1073/pnas.1804932115.

61. Zafar; Quarta; Marradi; Ragusa Recent Developments in the Reduction of Oxidative Stress through Antioxidant Polymeric Formulations. *Pharmaceutics* **2019**, *11*, 505, doi:10.3390/pharmaceutics11100505.

62. Kaneko, J.J. Carbohydrate Metabolism and Its Diseases. In *Clinical Biochemistry of Domestic Animals*; Elsevier, 1997; pp. 45–81 ISBN 978-0-12-396305-5.

63. Facucho-Oliveira, J.M.; St. John, J.C. The Relationship Between Pluripotency and Mitochondrial DNA Proliferation During Early Embryo Development and Embryonic Stem Cell Differentiation. *Stem Cell Rev and Rep* **2009**, *5*, 140–158, doi:10.1007/s12015-009-9058-0.

64. Al-Gubory, K.H.; Garrel, C.; Faure, P.; Sugino, N. Roles of Antioxidant Enzymes in Corpus Luteum Rescue from Reactive Oxygen Species-Induced Oxidative Stress. *Reproductive BioMedicine Online* **2012**, *25*, 551–560, doi:10.1016/j.rbmo.2012.08.004.

65. Stefanska, A.; Bergmann, K.; Sypniewska, G. Metabolic Syndrome and Menopause. In *Advances in Clinical Chemistry*; Elsevier, 2015; Vol. 72, pp. 1–75 ISBN 978-0-12-803314-2.

66. Fazio, F.; Arfuso, F.; Rizzo, M.; Giannetto, C.; Giudice, E.; Zanghì, E.; Piccione, G. Livestock Handling and Road Transport Influence Some Oxidative Stress Parameters in Ewes. *Journal of Veterinary Behavior* **2018**, *26*, 5–10, doi:10.1016/j.jveb.2018.03.010.

67. Wu, D.; Cederbaum, A.I. Alcohol, Oxidative Stress, and Free Radical Damage. *Alcohol Res Health* **2003**, *27*, 277–284.

68. Kostadinović, L.; Lević, J. Effects of Phytoadditives in Poultry and Pigs Diseases. *J Agron Technol Eng Manag* **2018**, *1*, 1–7.

69. Puvača, N.; LukaČ, D.; Ljubojević, D.; Stanaćev, V.; Beuković, M.; Kostadinović, Lj.; Plavša, N. Fatty Acid Composition and Regression Prediction of Fatty Acid Concentration in Edible Chicken Tissues. *Worlds Poult Sci J* **2014**, *70*, 585–592, doi:10.1017/S0043933914000634.

70. Schöttker, B.; Brenner, H.; Jansen, E.H.; Gardiner, J.; Peasey, A.; Kubínová, R.; Pająk, A.; Topor-Madry, R.; Tamosiunas, A.; Saum, K.-U.; et al. Evidence for the Free Radical/Oxidative Stress Theory of Ageing from the CHANCES Consortium: A Meta-Analysis of Individual Participant Data. *BMC Med* **2015**, *13*, 300, doi:10.1186/s12916-015-0537-7.

71. Celi, P. Biomarkers of Oxidative Stress in Ruminant Medicine. *Immunopharmacology and Immunotoxicology* **2011**, *33*, 233–240, doi:10.3109/08923973.2010.514917.

72. Sim, C.S.; Lee, J.-H.; Kim, S.H.; Han, M.W.; Kim, Y.; Oh, I.; Yun, S.-C.; Lee, J.C. Oxidative Stress in Schoolchildren with Allergic Rhinitis: Propensity Score Matching Case-Control Study. *Annals of Allergy, Asthma & Immunology* **2015**, *115*, 391–395, doi:10.1016/j.anai.2015.07.022.

73. Albera, E.; Kankofer, M. The Comparison of Antioxidative/Oxidative Profile in Blood, Colostrum and Milk of Early Post-Partum Cows and Their Newborns: Antioxidative/Oxidative Status in Post-Partum Cows and Calves. *Reproduction in Domestic Animals* **2011**, *46*, 763–769, doi:10.1111/j.1439-0531.2010.01737.x.

74. Abuelo, A.; Hernández, J.; Benedito, J.L.; Castillo, C. Oxidative Stress Index (OSi) as a New Tool to Assess Redox Status in Dairy Cattle during the Transition Period. *Animal* **2013**, *7*, 1374–1378, doi:10.1017/S1751731113000396.

75. Novac, C.S.; Andrei, S. The Impact of Mastitis on the Biochemical Parameters, Oxidative and Nitrosative Stress Markers in Goat's Milk: A Review. *Pathogens* **2020**, *9*, 882, doi:10.3390/pathogens9110882.

76. Güneş, M.; Camkurt, M.A.; Bulut, M.; Demir, S.; İbiloğlu, A.O.; Kaya, M.C.; Atlı, A.; Kaplan, İ.; Sir, A. Evaluation of Paraoxonase, Arylesterase and Malondialdehyde Levels in Schizophrenia Patients Taking Typical, Atypical and Combined Antipsychotic Treatment. *Clin Psychopharmacol Neurosci* **2016**, *14*, 345–350, doi:10.9758/cpn.2016.14.4.345.

77. Colakoglu, H.E.; Yazlik, M.O.; Kaya, U.; Colakoglu, E.C.; Kurt, S.; Oz, B.; Bayramoglu, R.; Vural, M.R.; Kuplulu, S. MDA and GSH-Px Activity in Transition Dairy Cows under Seasonal Variations and Their Relationship with Reproductive Performance. *Journal of Veterinary Research* **2017**, *61*, 497–502, doi:10.1515/jvetres-2017-0067.

78. Apak, R. Current Issues in Antioxidant Measurement. *J. Agric. Food Chem.* **2019**, *67*, 9187–9202, doi:10.1021/acs.jafc.9b03657.

79. Lin, C.-P.; Chuang, W.-C.; Lu, F.-J.; Chen, C.-Y. Anti-Oxidant and Anti-Inflammatory Effects of Hydrogen-Rich Water Alleviate Ethanol-Induced Fatty Liver in Mice. *World J Gastroenterol* **2017**, *23*, 4920–4934, doi:10.3748/wjg.v23.i27.4920.

80. Ahanger, M.A.; Aziz, U.; Alsahli, A.A.; Alyemeni, M.N.; Ahmad, P. Influence of Exogenous Salicylic Acid and Nitric Oxide on Growth, Photosynthesis, and Ascorbate-Glutathione Cycle in Salt Stressed Vigna Angularis. *Biomolecules* **2020**, *10*, 42, doi:10.3390/biom10010042.

81. Şenay, S.; Islim, P.; Tugay, A. Supplementation of Natural Antioxidants to Reduced Crude Protein Diets for Japanese Quails Exposed to Heat Stress. *Braz. J. Poult. Sci.* **2019**, *21*, eRBCA-2019-0694, doi:10.1590/1806-9061-2017-0694.

82. Teleanu, R.I.; Chircov, C.; Grumezescu, A.M.; Volceanov, A.; Teleanu, D.M. Antioxidant Therapies for Neuroprotection—A Review. *Journal of Clinical Medicine* **2019**, *8*, 1659, doi:10.3390/jcm8101659.

83. Ono, A.; Koshiyama, M.; Nakagawa, M.; Watanabe, Y.; Ikuta, E.; Seki, K.; Oowaki, M. The Preventive Effect of Dietary Antioxidants on Cervical Cancer Development. *Medicina* **2020**, *56*, 604, doi:10.3390/medicina56110604.

84. Pérez-Gálvez, A.; Viera, I.; Roca, M. Carotenoids and Chlorophylls as Antioxidants. *Antioxidants* **2020**, *9*, 505, doi:10.3390/antiox9060505.

85. Agarwal, A.; Sengupta, P.; Durairajanayagam, D. Role of L-Carnitine in Female Infertility. *Reprod Biol Endocrinol* **2018**, *16*, 5, doi:10.1186/s12958-018-0323-4.

86. Siotto, M.; Squitti, R. Copper Imbalance in Alzheimer's Disease: Overview of the Exchangeable Copper Component in Plasma and the Intriguing Role Albumin Plays. *Coordination Chemistry Reviews* **2018**, *371*, 86–95, doi:10.1016/j.ccr.2018.05.020.

87. Soderlund, D.M.; Clark, J.M.; Sheets, L.P.; Mullin, L.S.; Piccirillo, V.J.; Sargent, D.; Stevens, J.T.; Weiner, M.L. Mechanisms of Pyrethroid Neurotoxicity: Implications for Cumulative Risk Assessment. *Toxicology* **2002**, *171*, 3–59, doi:10.1016/s0300-483x(01)00569-8.

88. Rios, T.S.; Esqueda, M.T.S.-T.; Cruz, A.D.; Mora, J.L.C.; Perrusquía, R.G.; Morales, J.L.R.; Velasco, J.L.F.; Bautista, J.H. Oxidative State of Ewes with Different Number of Parity during Gestation and Lactation. *Pesq. Vet. Bras.* **2017**, *37*, 1405–1410, doi:10.1590/s0100-736x2017001200008.

89. Surai, P.F.; Kochish, I.I. Antioxidant Systems and Vitagenes in Poultry Biology: Heat Shock Proteins. In *Heat Shock Proteins in Veterinary Medicine and Sciences*; Asea, A.A.A., Kaur, P., Eds.; Heat Shock Proteins; Springer International Publishing: Cham, 2017; Vol. 12, pp. 123–177 ISBN 978-3-319-73376-0.

90. Rout, P.K.; Behera, B.K. Factors Influencing Livestock Way of Life. In *Sustainability in Ruminant Livestock*; Springer Singapore: Singapore, 2021; pp. 117–136 ISBN 978-981-334-342-9.

91. Erişir, M.; Benzer, F.; Kandemir, F.M. Changes in the Rate of Lipid Peroxidation in Plasma and Selected Blood Antioxidants before and during Pregnancy in Ewes. *Acta Vet. Brno* **2009**, *78*, 237–242, doi:10.2754/avb200978020237.

92. Shi, L.; Xu, Y.; Mao, C.; Wang, Z.; Guo, S.; Jin, X.; Yan, S.; Shi, B. Effects of Heat Stress on Antioxidant Status and Immune Function and Expression of Related Genes in Lambs. *Int J Biometeorol* **2020**, *64*, 2093–2104, doi:10.1007/s00484-020-02000-0.

93. Kesavulu, M.M.; Rao, B.K.; Giri, R.; Vijaya, J.; Subramanyam, G.; Apparao, C. Lipid Peroxidation and Antioxidant Enzyme Status in Type 2 Diabetics with Coronary Heart Disease. *Diabetes Research and Clinical Practice* **2001**, *53*, 33–39, doi:10.1016/S0168-8227(01)00238-8.

94. Sakha M; Anoushepour A; Nadalian M.g; Khaki Z EFFECT OF INDUCTION OF SUBCLINICAL PREGNANCY TOXEMIA ON SERUM CERULOPLASMIN LEVELS IN EWE. *JOURNAL OF COMPARATIVE PATHOBIOLOGY IRAN* **2013**, *10*, 949–954.

95. Sanz-Fernandez, M.V.; Daniel, J.-B.; Seymour, D.J.; Kvidera, S.K.; Bester, Z.; Doelman, J.; Martín-Tereso, J. Targeting the Hindgut to Improve Health and Performance in Cattle. *Animals* **2020**, *10*, 1817, doi:10.3390/ani10101817.

96. Giorgio, D.; Di Trana, A.; Di Gregorio, P.; Rando, A.; Avondo, M.; Bonanno, A.; Valenti, B.; Di Grigoli, A. Oxidative Status of Goats with Different CSN1S1 Genotypes Fed Ad Libitum with Fresh and Dry Forages. *Antioxidants* **2020**, *9*, 224, doi:10.3390/antiox9030224.

97. Amer, H.; Donia, G.; Ibrahim, N. Oxidative Stress and Trace Elements Status in Different Reproductive Stages of Shami Goat Does Fed Salt-Tolerant Plants under Semi-Arid Conditions in Egypt. *Journal of Animal and Poultry Production* **2020**, *11*, 109–116, doi:10.21608/jappmu.2020.87134.

98. Trevisi, E.; Minuti, A. Assessment of the Innate Immune Response in the Periparturient Cow. *Research in Veterinary Science* **2018**, *116*, 47–54, doi:10.1016/j.rvsc.2017.12.001.

99. Türkeş, C.; Beydemir, Ş. Inhibition of Human Serum Paraoxonase-I with Antimycotic Drugs: In Vitro and In Silico Studies. *Appl Biochem Biotechnol* **2020**, *190*, 252–269, doi:10.1007/s12010-019-03073-3.

100. Abbas, S.; Yousaf, M.S.; Ahmad, I.; Rehman, H.; Mahmood, A.K.; Ashraf, S.; Blouch, R.S.; Shahzad, A.H.; Nak, Y.; Nak, D. Evaluation of Serum Analytes in Pregnant and Non-Pregnant Dairy Cows as Indicators of Pregnancy. *SA J. An. Sci.* **2021**, *50*, doi:10.4314/sajas.v50i5.15.

101. Gross, J.J.; Bruckmaier, R.M. Invited Review: Metabolic Challenges and Adaptation during Different Functional Stages of the Mammary Gland in Dairy Cows:

Perspectives for Sustainable Milk Production. *Journal of Dairy Science* **2019**, *102*, 2828–2843, doi:10.3168/jds.2018-15713.

102. Merghem, M.; Dahamna, S.; Khennouf, S. Acute Toxicity Study of Aqueous Extracts of Thymelaea Hirsuta Leaves And Flowers in Rabbits. *J. Drug Delivery Ther.* **2020**, *10*, 67–69, doi:10.22270/jddt.v10i4.4141.

103. Tibbo, M.; Woldemeskel, M.; Aragaw, K.; Rege, J.E.O. Serum Enzyme Levels and Influencing Factors in Three Indigenous Ethiopian Sheep Breeds. *Comp Clin Pathol* **2008**, *17*, 149–155, doi:10.1007/s00580-008-0735-1.

104. Luz, G.B.; Maffi, A.S.; Xavier, E.G.; Correa, M.N.; Gasperin, B.G.; Brauner, C.C. Induction of Lactation in Dairy Heifers: Milk Production, Inflammatory and Metabolic Aspects. *Arq. Bras. Med. Vet. Zootec.* **2020**, *72*, 371–378, doi:10.1590/1678-4162-11246.

105. Xu, Q.; Li, Y.; Gao, X.; Kang, K.; Williams, J.G.; Tong, L.; Liu, J.; Ji, M.; Deterding, L.J.; Tong, X.; et al. HNF4α Regulates Sulfur Amino Acid Metabolism and Confers Sensitivity to Methionine Restriction in Liver Cancer. *Nat Commun* **2020**, *11*, 3978, doi:10.1038/s41467-020-17818-w.

106. Ullah, R.; Khan, M.; Shah, S.A.; Saeed, K.; Kim, M.O. Natural Antioxidant Anthocyanins—A Hidden Therapeutic Candidate in Metabolic Disorders with Major Focus in Neurodegeneration. *Nutrients* **2019**, *11*, 1195, doi:10.3390/nu11061195.

107. Piccione, G.; Casella, S.; Giannetto, C.; Bazzano, M.; Giudice, E.; Fazio, F. Oxidative Stress Associated with Road Transportation in Ewes. *Small Ruminant Research* **2013**, *112*, 235–238, doi:10.1016/j.smallrumres.2012.11.001.

108. Kanakkaparambil, R.; Singh, R.; Li, D.; Webb, R.; Sinclair, K.D. B-Vitamin and Homocysteine Status Determines Ovarian Response to Gonadotropin Treatment in Sheep1. *Biology of Reproduction* **2009**, *80*, 743–752, doi:10.1095/biolreprod.108.072074.

109. Feringa, H.H.H.; Laskey, D.A.; Dickson, J.E.; Coleman, C.I. The Effect of Grape Seed Extract on Cardiovascular Risk Markers: A Meta-Analysis of Randomized Controlled Trials. *Journal of the American Dietetic Association* **2011**, *111*, 1173–1181, doi:10.1016/j.jada.2011.05.015.

110. Yokus, B.; Cakir, D.U.; Kanay, Z.; Gulten, T.; Uysal, E. Effects of Seasonal and Physiological Variations on the Serum Chemistry, Vitamins and Thyroid Hormone Concentrations in Sheep. *J Vet Med Series A* **2006**, *53*, 271–276, doi:10.1111/j.1439-0442.2006.00831.x.

111. Beura, S.S.; Pradhan, C.R.; Panigrahi, B.; Sahoo, C.; Sahoo, A.; Jena, B. Effect of Balanced Concentrate Ration on the Performance and Hematobiochemical Profile

of Lactating Native Ewes and Lambs in Coastal Odisha. *Veterinary World* **2014**, 1047–1057.

112. Baumgard, L.H.; Rhoads, R.P. Effects of Heat Stress on Postabsorptive Metabolism and Energetics. *Annu. Rev. Anim. Biosci.* **2013**, *1*, 311–337, doi:10.1146/annurev-animal-031412-103644.

113. Kachhawa, J.P.; Kumar, S.; Sharma, A.; Singh, A.P.; Ahuja, A. Studies on Alterations of Clinical and Hemato-Biochemical Parameters before and after Treatment in Calves Naturally Infected with Theileriosis. *Vet World* **2016**, *9*, 1381–1385, doi:10.14202/vetworld.2016.1381-1385.

114. Belkacem, L.; Safsaf, B.; Tlidjane, M.; Loughraieb, S.; Belkadi, S. Steroid Hormones and Energetic Metabolites Profiles in Ewes Raised under Arid and Semi-Arid Climates of Algeria. *Biological Rhythm Research* **2019**, *50*, 845–856, doi:10.1080/09291016.2018.1499373.

115. Rutkowski, M.; Grzegorczyk, K. Modifications of Spectrophotometric Methods for Antioxidative Vitamins Determination Convenient in Analytic Practice. *Acta Scientiarum Polonorum Technologia Alimentaria* **2007**, *6*.

116. Turk, R.; Juretić, D.; Gereš, D.; Svetina, A.; Turk, N.; Flegar-Meštrić, Z. Influence of Oxidative Stress and Metabolic Adaptation on PON1 Activity and MDA Level in Transition Dairy Cows. *Animal Reproduction Science* **2008**, *108*, 98–106, doi:10.1016/j.anireprosci.2007.07.012.

117. Szasz, G. A Kinetic Photometric Method for Serum γ-Glutamyl Transpeptidase. *Clinical Chemistry* **1969**, *15*, 124–136, doi:10.1093/clinchem/15.2.124.

118. Nawito, M.F.; Hameed, A.R.A.E.; Sosa, A.S.A.; Mahmoud, K.G.M. Impact of Pregnancy and Nutrition on Oxidant/Antioxidant Balance in Sheep and Goats Reared in South Sinai, Egypt. *Vet World* **2016**, *9*, 801–805, doi:10.14202/vetworld.2016.801-805.

119. Lindsay, J.R.; Nieman, L.K. The Hypothalamic-Pituitary-Adrenal Axis in Pregnancy: Challenges in Disease Detection and Treatment. *Endocrine Reviews* **2005**, *26*, 775–799, doi:10.1210/er.2004-0025.

120. Dizdar, E.A.; Uras, N.; Oguz, S.; Erdeve, O.; Sari, F.N.; Aydemir, C.; Dilmen, U. Total Antioxidant Capacity and Total Oxidant Status after Surfactant Treatment in Preterm Infants with Respiratory Distress Syndrome. *Annals of Clinical Biochemistry* **2011**, *48*, 462–467, doi:10.1258/acb.2011.010285.

121. Entringer, S.; Buss, C.; Shirtcliff, E.A.; Cammack, A.L.; Yim, I.S.; Chicz-DeMet, A.; Sandman, C.A.; Wadhwa, P.D. Attenuation of Maternal Psychophysiological Stress Responses and the Maternal Cortisol Awakening Response over the Course of Human Pregnancy. *Stress* **2010**, *13*, 258–268, doi:10.3109/10253890903349501.

122. Gür, S.; Türk, G.; Demirci, E.; Yüce, A.; Sönmez, M.; Özer, Ş.; Aksu, E. Effect of Pregnancy and Foetal Number on Diameter of Corpus Luteum, Maternal Progesterone Concentration and Oxidant/Antioxidant Balance in Ewes: Pregnancy Affects Maternal Oxidative Stress. *Reproduction in Domestic Animals* **2011**, *46*, 289–295, doi:10.1111/j.1439-0531.2010.01660.x.

123. Dias e Silva, T.P.; Costa Torreão, J.N. da; Torreão Marques, C.A.; de Araújo, M.J.; Bezerra, L.R.; kumar Dhanasekaran, D.; Sejian, V. Effect of Multiple Stress Factors (Thermal, Nutritional and Pregnancy Type) on Adaptive Capability of Native Ewes under Semi-Arid Environment. *Journal of Thermal Biology* **2016**, *59*, 39–46, doi:10.1016/j.jtherbio.2016.05.001.

124. Adikwu, E.; Deo, O. Hepatoprotective Effect of Vitamin C (Ascorbic Acid). *PP* **2013**, *04*, 84–92, doi:10.4236/pp.2013.41012.

125. Charismiadou, M.A.; Bizelis, J.A.; Rogdakis, E. Metabolic Changes during the Perinatal Period in Dairy Sheep in Relation to Level of Nutrition and Breed. I. Late Pregnancy. *J Anim Physiol Anim Nutr* **2000**, *84*, 61–72, doi:10.1046/j.1439-0396.2000.00282.x.

126. Abdollahi, E.; Kohram, H.; Shahir, M.H. Plasma Concentrations of Essential Trace Microminerals and Thyroid Hormones during Single or Twin Pregnancies in Fat-Tailed Ewes. *Small Ruminant Research* **2013**, *113*, 360–364, doi:10.1016/j.smallrumres.2013.03.019.

127. Guller, S.; Buhimschi, C.S.; Ma, Y.Y.; Huang, S.T.J.; Yang, L.; Kuczynski, E.; Zambrano, E.; Lockwood, C.J.; Buhimschi, I.A. Placental Expression of Ceruloplasmin in Pregnancies Complicated by Severe Preeclampsia. *Lab Invest* **2008**, *88*, 1057–1067, doi:10.1038/labinvest.2008.74.

128. Sun, L.; Guo, Y.; Fan, Y.; Nie, H.; Wang, R.; Wang, F. Metabolic Profiling of Stages of Healthy Pregnancy in Hu Sheep Using Nuclear Magnetic Resonance (NMR). *Theriogenology* **2017**, *92*, 121–128, doi:10.1016/j.theriogenology.2017.01.025.

129. Antunović, Z.; Šperanda, M.; Steiner, Z. The Influence of Age and the Reproductive Status to the Blood Indicators of the Ewes. *Arch. Anim. Breed.* **2004**, *47*, 265–273, doi:10.5194/aab-47-265-2004.

130. Sajid, M.; Younus, M.; Khan, M.-R.; Anjum, A.A.; Arshad, M.; Ehtisham-ul-Haque, S.; Rafique, M.; Idrees, M. Assessing Lead (Pb) Residues in Lohi Sheep and Its Impact on Hematological and Biochemical Parameters. *Pol. J. Environ. Stud.* **2018**, *27*, 1717–1723, doi:10.15244/pjoes/76181.

131. Damián, J.P.; de Soto, L.; Espindola, D.; Gil, J.; van Lier, E. Intranasal Oxytocin Affects the Stress Response to Social Isolation in Sheep. *Physiology & Behavior* **2021**, *230*, 113282, doi:10.1016/j.physbeh.2020.113282.

132. Ahmed, M.H.; Wilkens, M.R.; Ganter, M.; Breves, G. Serum Parameters Related to Mineral Homeostasis and Energy Metabolism in Ewes Kept on Different Dietary Magnesium Supply during the Transition Period. *Research in Veterinary Science* **2021**, *134*, 19–26, doi:10.1016/j.rvsc.2020.11.016.

133. Stewart, W.C.; Murphy, T.W.; Page, C.M.; Rule, D.C.; Taylor, J.B.; Austin, K.; Pankey, C. Effects of Increasing Dietary Zinc Sulfate Fed to Primiparous Ewes: I. Effects on Serum Metabolites, Mineral Transfer Efficiency, and Animal Performance*. *Applied Animal Science* **2020**, *36*, 839–850, doi:10.15232/aas.2020-02057.

ENCLOSURE 1

Authorship statement

Signed: **TAMER TASHLA**, M.Sc

I declare

that the doctoral dissertation entitled: „**OXIDATIVE STRESS DURING PREGNANCY AND LACTATION OF LOHI SHEEPS**“ the result of my own research work, that the proposed dissertation in whole and in parts was not proposed for obtaining any diploma according to the study programs of other higher education institutions, that the results were correctly stated and that I did not infringe copyright and use the intellectual property of others.

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ENCLOSURE 2

Statement on the identity of the printed and electronic version of the doctoral thesis

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Thesis title: „**OXIDATIVE STRESS DURING PREGNANCY AND LACTATION OF LOHI SHEEPS**“

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Signed

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I declare that the printed version of my doctoral thesis is identical to the electronic version that I submitted for publication on the Digital Library of the Dissertations portal of the University Business Academy in Novi Sad.

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ENCLOSURE 3

Изјава о коришћењу

(*in Serbian language*)

Овлашћујем Централну библиотеку Универзитета Привредна академија у Новом Саду да у Дигиталну библиотеку дисертација Универзитета унесе моју докторску дисертацију под насловом: „**OXIDATIVE STRESS DURING PREGNANCY AND LACTATION OF LOHI SHEEPS**“

Дисертацију са свим прилозима предао сам у електронском формату погодном за трајно архивирање.

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У Новом Саду, _____ Потпис

Tamer Tashla

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