EFFECT OF NICKEL SUPPLEMENTATION ON GROWTH PERFORMANCE, ANTIOXIDANT AND BLOOD METABOLITES OF MUZAFFARNAGRI SHEEP

THESIS

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SARDAR VALLABHBHAI PATEL UNIVERSITY OF AGRICULTURE AND TECHNOLOGY MEERUT – 250110 (U.P.) INDIA

BY

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CERTIFICATE

This is to certify that the thesis entitled "**Effect of nickel supplementation on growth performance, antioxidant and blood metabolites of Muzaffarnagri sheep"** submitted in partial fulfillment of the requirements for the degree of **Master of Science in Agriculture** with major in **Animal Husbandry** of the college of Post Graduate Studies, **Sardar Vallabhbhai Patel University of Agriculture & Technology Meerut** is a record of bona-fide research carried out by **Mr. Amit Kumar Gangwar, Id. No. 4820** under my supervision and no part of the thesis has been submitted for any other degree or diploma.

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We, the undersigned, members of the Advisory Committee of **Mr. Amit Kumar Gangwar, Id. No. 4820,** a candidate for the degree of **Master of Science in Agriculture** with major in **Animal Husbandry** agree that the thesis entitled **"Effect of nickel supplementation on growth performance, antioxidant and blood metabolites of Muzaffarnagri sheep"** may be submitted by **Mr. Amit Kumar Gangwar** in partial fulfillment of the requirements for the degree.

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Member	Member	Member	Member

ABBREVIATIONS

%	Percent
<	Less than
>	More than
ºC	Degree centigrade
e.g.	For Example
ºF	Degree Fahrenheit
et al	Ethically all (and others)
etc.	And so forth
i.e.	That is
@	At the rate of
pH	Negative logarithm of hydrogen ion
Fig.	Figure
Kg	Kilo gram
g	Gram
g/ml	Grams per milliliter
mg	Milligram
mg/kg	Milligram per kilogram
mg/l	Milligram per liter
mg/dl	Miligram per deciliter
mg/ml	Mili gram per milli lite
PPM	Part per million
PPB	Part per Billion
ml	Milliliter
L	Liter
µl	Microliter
µM	Micro mole
ml/g	Milliliter per gram
N	Normal
mM	Milli mole
µ mol/l	Micro mole per liter
IU/L	International unit per liter
IU/ml	International unit per milliliter

H_2O	Water
ng/ µl	Nanogram/microliter
hr	Hours
min.	Minute
Sec.	Second
T0	Treatment1
T1	Treatment2
T2	Treatment3
DM	Dry matter
OM	Organic matter
EE	Ether extract
TA	Total ash
CF	Crude fiber
CP	Crude protein
NFE	Nitrogen free extract
Hb	Hemoglobin
TDN	Total digestible nutrient
ARC	Agricultural Research Council
NRC	National Research Council
ICAR	Indian Council of Agriculture Research
DW	Dry weight
DMI	Dry matter intake
% BW	Percent body weight
g/kg $W^{0.75}$	Gram per kg metabolic body weight
THI	Temperature humidity index
ADG	Average daily gain
FRAP	Ferric reducing antioxidant power
SOD	Superoxide dismutase
HDL	High Density Lipoprotein
BUN	Blood urea nitrogen
SVPUAT	Sardar VallabhBhai Patel University of Agriculture & Technology Meerut-250 110 (U.P), INDIA
UP	Uttar Pradesh

Conc.	Concentrate
$CaCl_2$	Calcium chloride
NaCl	Sodium chloride
NaOH	Sodium hydro-oxide
$MgSO_4$	Magnesium sulphate
$CoCl_2$	Cobalt chloride
KI	Potassium iodide
$CuSO_4$	Copper sulphate
$ZnSO_4$	Zinc sulphate
$FeCl_2$	Ferrous chloride
$MnCl_2$	Manganese chloride
KNO_3	Potassium nitrate
HNO_3	Nitric acid
$HClO_4$	Perchloric acid
HCl	Hydrochloric acid
H_2SO_4	Sulphuric acid
Na_2HPO_4	Di-sodium hydrogen phosphate
Ca	Calcium
$KMNO_4$	Potassium permanganate
Cu	Copper
Zn	Zinc
Fe	Iron
Mn	Manganese
Co	Cobalt
Mo	Molybdenum
I	Iodine
Cr	Chromium
Ca	Calcium
P	Phosphorus
CO_2	Carbon di oxide
PF	Partitioning factor
FCR	Feed conversion ratio
BCS	Body condition score

TIg	Total immunoglobulin
OD	Optical density
ELISA	Enzyme linked immunosorbant assay
PCV	packed cell volume
BUN	Blood urea nitrogen

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CHAPTER - 1 INTRODUCTION

All Minerals essential animals need for growth and development, as essential components of many enzymes, vitamins, and respiratoet pigments, or as cofactors in metabolism, catalysts and enzyme activators, immunity and reproduction. Both macro and micro minerals play a vital role in augmenting production and reproduction in farm animals. The severity of the minerals deficiency in farm animals depends upon the type of diet, age physiological status of the animals and the agro-climatic conditions.

Apart from the well established role of macro and trace minerals, evidence has been presented suggesting that a number of trace elements like Nickel (Ni), Vanadium (V), Boron (B) Lithium (Li) Molybdenum (Mo), Rubidium (Rb) Chromium (Cr), and Silicon (Si), not previously recognized as essential, are required at least by certain animals species **(Nielsen, 1980)**. These elements are grouped as newer essential trace elements or occasionally beneficial elements or probable essential minerals because deprived animals were unhealthy and showed physiological response to the supplementation probable essential for maintaining health, immunity, growth, production and reproduction. Occasionally beneficial elements are very important for various cell functions at biological chemical and molecular levels.

Livestock systems have both positive and negative effects on the natural resource base, public health, social equity and economic growth Currently, livestock is one of the fastest growing agricultural subsectors in developing countries. Its share of agricultural GDP is already 33 per cent and is quickly increasing. This growth is driven by the rapidly increasing demand for livestock products, this demand being driven by population growth, urbanization and increasing incomes in developing countries. India's livestock

sector has continuously provided structural support to the rural economy as an important vocation for the rural population, next only crop rising. On account of favorable socio-economic factors such as changing eating habits, higher purchasing power, urbanization, increasing health consciousness towards protein-rich diet, preferred meat due to religious preferences, there has been an increase in demand for meat and the sector has gained importance in terms of contribution to income, employment and foreign exchange earnings.

Small ruminants play an important role in the Indian economy and it provides livelihood to two-third of the rural community. Sheep are important species of livestock for India. They contribute greatly to the agrarian economy, especially in areas where crop and dairy farming are not economical and play an important role in the livelihood of a large proportion of small and marginal landless labor.

Muzaffarnagari Sheep with its multi-facet utility for wool, meat, milk, skins and manure, form an important component of the rural economy particularly in the arid, semi-arid and mountainous areas of the country. It provides a dependable source of income to the shepherds through the sale of wool and animals. They play an important role in the livelihood of a large percentage of small and marginal farmers and landless laborers engaged in sheep rearing.

Nickel (Ni) plays an important role and has been lesser studied in the animal. It has been reported that Ni may have a role in the enzymes and hormone activation, oxidative stress and immunity and in the regulation of carbohydrate, protein and lipid metabolism. It containing superoxide dismutase (Ni-SOD) is a metalloid enzyme that, like the other SODs, protects cells from oxidative damage by catalyzing the cytotoxic

superoxide radicals to hydrogen peroxide and molecular oxygen. Ni may also act to stimulate or inhibit the release of various hormones. At low concentration, Ni specifically inhibited prolactin release from the pituitary but at higher concentrations, stimulate the release of growth hormone, thyrotropin, luteinizing, follicle-stimulating and adrenocorticotropic hormones from the bovine pituitary **(La Bella *et al*; 1973** a & b**)**. Ni supplementation changes the ratio of VFA production in the rumen, but the ratio has been inconsistent. Ni functions either as a cofactor or structural component in specific metallo-enzymes or metalloproteins or as a bio-ligand cofactor facilitating the intestinal absorption of various minerals. Therefore interactions have been reported between Ni and iron (Fe), copper (Cu), zinc (Zn) and some other trace minerals in the body**.** Ni deprivation resulted in lowered activities of a number of liver enzymes as well decreased concentration of triglycerides, glucose and glycogen in the liver and decreased concentration of urea and glucose in serum (**Schnegg and Krichgessner, 1975).** Ni deficiency resulted in histological and biochemical changes, reduced Fe respiration, depressed growth, hematocrit hemoglobin concentration, erythrocyte counts.

Ni low doses of some heavy metals can improve immune system function, whereas higher doses are suppressive. Although there are some reports of Ni as heavy metals affecting immune system function, the causal mechanisms of Ni in immunomodulation are unclear. Ni possesses insulin-like activity (induced hypo insulinemic response) on fat cell membranes in rats, with stimulation of glucose incorporation and diminution of lipolysis. Nickel is an arising fundamental minor component. The vitality of nickel is currently commonly acknowledged, in light of the different side effects brought about by nickel insufficiency in various creatures. It is

found in most noteworthy focuses in lung, kidney and some chemical creating tissues. Modification in nickel focus influences the creation and activity of certain chemicals like prolactin, adrenaline, noradrenaline and aldosterone. Inside cells, nickel changes film properties and impacts oxidation decrease frameworks. It has an incredible fondness for cell structures like chromosomes and particle channels.

Nickel is a constituent piece of all organs of vertebrates. Its retention can be controlled. Low nickel offers to lessen development; this is especially valid for intra-uterine turn of events. Such offers likewise decline the future of replicating creatures. Nickel inadequacy is joined by histological and biochemical changes and decreased iron resorption and prompts pallor. It can upset the joining of calcium into a skeleton and lead to parakeratosis-like harm, which discovers articulation in upset zinc digestion. Nickel insufficiency brings about lower exercises of various dehydrogenases and transaminases and, most importantly, of alpha-amylase, and especially influences starch digestion. A checked reduction in digestion was seen on account of the energy sources fat, glucose, and glycogen. Nickel hence plays out a fundamental capacity in digestion: it is a fundamental component. The nickel necessities of people and creatures add up to under 500 micrograms/kg and are likely even extensively lower. It subsequently follows that considering the accessible nickel offer; essential nickel insufficiency in people and creatures can be avoided, basically in the current situation with information.

Nickel is ubiquitous in the biosphere. Nickel introduced into the environment from natural or human sources is circulated through the system by chemical and physical processes and through biological transport mechanisms of living organisms **(National Academy of Sciences [NAS] 1975; WHO 1991**). Nickel is essential for the normal

growth of many species of microorganisms and plants and several species of vertebrates, including chickens, cows, goats, pigs, rats, and sheep **(NAS 1975; USEPA 1980; WHO 1991; USPHS 1993**). Water contamination and industrial pollution by fluoride (F) are considered among one the most common environmental problems. Acute or chronic exposure to high levels of F results in adverse health effects. In vivo studies show that F causes kidney and nerve damage and is also the cause of reproductive toxicity in humans and animals. Previous studies indicated that chronic ingestion of excessive F damages the teeth and bones, causing various types of undesirable alterations and disorders in the functions of non-skeletal organs and body systems.

Nickel is a potent animal teratogen. Inhalation and exposure of nickel carbonyl compounds to rats and hamsters were found to cause fetal death, decreased weight gain and eye malformations Nickel was also proved to be embryo lethal and teratogenic to white leghorn strains of the domestic chicken (*Gallus* sp.), possibly due to the mitosis - inhibiting the activity of nickel compounds. Malformations might include poorly developed or missing brain and eyes, everted viscera, short and twisted neck and limbs, hemorrhaging and reduction in body size **(Gilani and Marano, 1980).** Rodents exposed to nickel during gestation showed a decline in the frequency of implantation of fertilized eggs, enhanced resorption of fertilized eggs and fetus, an increased frequency of stillbirths and growth abnormalities in live-born young. Albeit the atomic systems by which nickel intensifies cause malignant growth are as yet under extraordinary examination, the cancer-causing activities of nickel compounds are thought to include oxidative pressure, genomic DNA harm, epigenetic impact and the guideline of quality

articulation by initiation of certain record factors identified with relating signal transduction pathways**.**

Objectives

- To observe the impact of nickel supplementation on growth performance of Muzaffarnagari sheep.
- To find out the impact of nickel supplementation on antioxidant status of Muzaffarnagari sheep.
- To study the impact of nickel supplementation on blood metabolites of Muzaffarnagari sheep.

Minerals provide the essential nutrients animals need for growth and development, an essential part of many enzymes, hormones, or functions as cofactors or bio-ligand in metabolism, catalysts and enzyme activation. Even moderate deficiencies of minerals can adversely impact animal health and performance. Until 1950, mineral elements were classified as essential: these comprise the major elements and the micro or trace elements. By 1970, molybdenum, selenium, chromium and fluorine had been added to the list of trace elements. The role of major and trace minerals in the performance of livestock is well established. Recently some other trace elements like Ni have been identified which also have a certain beneficial role in animals. These elements are grouped as newer essential trace elements because deprived animals were unhealthy and showed physiological responses to the supplementation.

Chemical properties of nickel

The name Ni comes from the German word Kupfer nickel, meaning "Old Nick's copper," a term used by German miners. Swedish mineralogist Axel Fredrik Cronstedt (1722-65) was the first person to realize that Ni was a new element. He found something in the mineral that did not act like cobalt, copper, or any other known element. He used a shortened version of Kupfer nickel for the name of the new element and called it as Ni has an atomic number of 28, an atomic mass of 58.69 and exists in two oxidation states (+2 and +3) and five naturally occurring isotopes (58Ni, 60Ni, 61Ni, 62Ni and 64Ni). It is a silvery-white siderophile metallic element with chalcophilic and lithophilic affinities and forms several minerals, including

pentlandite, Niine and ullmannite. Ni forms compounds in several oxidation states, the divalent ion seems to be the most important for both organic and inorganic substances, but the trivalent form may be generated by redox reactions in the cell. Divalent (Ni2+) ion is intermediate in size (69 pm) between Mg 2+ and Ni2+ (72 and 61 pm respectively), for which it substitutes during fractionation, and it is partitioned into ferromagnesian minerals such as olivine, orthopyroxene and spinel. Ni is highly mobile under acidic and oxidizing conditions. In natural water, Ni may exist in one of three oxidation states (+2, +3 and +4), although the free ion Ni predominates. Chloride, nitrate and sulphate compounds of Ni are very soluble in water, but nickel carbonate (NiCO3) and, in particular, nickel hydroxide {Ni(OH)2} and nickel phosphate {Ni3(PO4)2} are insoluble. Colloidal nickel hydroxide is present above pH 8 and under reducing conditions, Ni is incorporated into sulphides, such as millerite (NiS), also lowering its mobility Ni forms complexes with adenosine triphosphate (ATP), amino acids, peptides, proteins and deoxyribonucleic acid in the biological system.

2.1 Impact of nickel supplementation on growth performance

2.1.1 Body weight (BW) and body weight gain (BWG)

O'Dell *et al.* (1970) found that the impact of nickel supplementation in cattle shows that daily nickel intake of 500 mg as either nickelous chloride or nickelous carbonate administered in gelatin capsule greatly reduced voluntary feed intake and were highly toxic.

Anke *et al.* (1977) reported that the impact of nickel in the goat resulted revealed that goats receiving a diet containing 1 ppm Ni gained 21% slower than animals fed with the basal diet supplemented along with 10 ppm Ni.

Kirchgessner and Roth (1977) found that Ni supplementation at 125-, 250-, or 375-ppm levels had a non-significant ($p < 0.05$) effect whereas, at 500-level ppm depressed feed intake and growth performance in the pig.

Graham *et al.* 1978 report that the influence of nickel in mice which pointed that the intramuscularly exposed mice indicated that concentrations greater than or equal to 3.90 µg of Ni/g body weight (as $NiSO_4$) and greater than or equal to 9.25 µg of Ni/g body weight (as $NiCl_2$) resulted in significant immune suppression.

Spears *et al.* (1979) conduct that to determine nickel will supplement the basal diets at a level of 0 or 5 ppm in lambs. The average daily gain will significantly increase while serum urea- nitrogen and total serum proteins shall decrease by nickel in the first period.

Smialowicz *et al.* (1987) conduct that to know the nickel doses ranging from 10 to 20mg/kg in the rats. Significant ($P < 0.05$) decreases in body weights shall observe in rats injected with 15 and 20 mg/kg $NiCl_2$ as shall decreases in spleen weights of rats receiving 20 mg/kg.

Dunnick J. K. *et al.* (1989) reported that to study of nickel supplementation on the rats after inhalation exposure concentrations used (as mg Ni/m3) shall 0.4–7.9 for Ni, 0.02–0.4 for $NiSO_4 \cdot 6H_2O$, and 0.11–1.8 for Ni_3S_2. No exposure-related effects on mortality and only minor effects on body weight gain shall see in rats or mice.

Milne *et al.* (1990) work in the United States of America has shown that dietary supplements of nickel (Ni) can result increase growth rate in lambs and steers given low protein diets.

Smith *et al.* (1993) report suggested that soluble nickel salts may affect development in rats. Pup birth weight gain will reduce only in male pups exposed to 50 ppm Ni during L1. They conclude that 10 ppm Ni represents the lowest observed level.

Pandey *et al*. (1999) found that there is no change in the body weight of mice orally administered by NiSO4. And another is that higher DMI in 3.0 ppm Ni supplemented heifers might be due to better digestibility of nutrients in Ni supplemented group.

Wilson *et al.* (2001) conduct that nickel supplementation in the 6 weeks of age, the shear fracture energy of the tibia from the caged bird's increases when the basal diet supplementation with 25 mg of dietary nickel per kilogram of feed. Dietary nickel did not affect bird body weight, but the caged broilers (2161 g) shall heavier than the floor birds (2005 g). Nickel does not affect the strength characteristics of the tibia from the floor birds.

Berseny *et al.* (2004) conduct that to deem the supplementary of nickel in broiler chicken experiments shall carry out to study the effects of nickel (Ni) supplementation of 50 mg Ni/kg slightly improved the body weight gain (BWG) in broiler chickens. However, Ni added at a level of 500 mg/kg significantly ($P < 0.05$) reduces the BWG by 10% and results in significantly ($P < 0.05$) worse (2.3 ± 0.2 kg/kg) FCE.

Samal and Mishra (2011) found that to know the nickel is given to body weight gain significantly reduced in weanling rats exposed to nickel (as nickel acetate) at concentrations of 500 or 1000 mg/kg in the diet (equivalent to 25 or 50 mg/kg of body

weight per day) for 6 weeks compared with controls. No effects shall observe in rats exposed to 100 mg/kg in the diet (equivalent to 5 mg/kg of body weight per day).

Gathwan *et al.* (2013) report a study is to evaluate the toxic effects of nickel (Ni) on the liver structure of male rats. Male mice weighing 30–32 g, 50 days old, shall be treated with 1–16 mg/kg (body wt.) NiCl2. Liver weight and body weight decreased with increasing dose.

Haneen and Amel (2020) report that to determine the supplementary of nickel in the body weight gain of the pregnant rat show decreases in 14 days of pregnancy in the treated group as compared with the control group while in 12 days of pregnancy showed increases in the treated group as compared with control.

2.2 Effect of nickel supplementation on feed intake of Muzaffarnagri sheep

2.2.1 Feed consumption (FC) and Feed conversion ratio (FCR)

Feed consumption in a growing animal changes as its size increases. **Pittroff and Kothmann (2001).** The animal size was present in all, but breeds in only one. The models also used different expressions for animal size, including BW, BW 0.75, and BW 0.73. Intake of different sheep breeds at a BW may vary with mature BW; called **A. Taylor (1980)** proposed 2 genetic size-scaling rules. The first was to treat all time variables, such as daily feed intake, as proportional to A0.73. The second was to express BW as a proportion. Actual intakes for different kinds of sheep over a range of BW on different feeds will be presented here. These may be used to test models of feed consumption.

Weber and Reid (1968) in this study, we know the nickel supplementation @ of 1100 ppm nickel incorporated diet to delineate the effects associated with feed

consumption and nickel toxicity in the growing chicks. No significant differences in growth rate shall obtain with 1100 ppm nickel.

Kondakov M. P. (1970) found that a stimulation increase in intake of Ni and deficiency in sheep and caused morphological changes in the organs to become more pronounced, especially in organs with high content of Nickel. Even the Ni in the ratio, reduced accumulation of Ni and morphological change in the organ become less.

O'Dell *et al*. (1970) study the preliminary experiment with dairy calves ascertained the effects of supplemental nickel on voluntary feed consumption and animal health observed as nickel in the diet increased from 50 to 100 to 200 ppm as nickel us chloride and from 250 to 500 to 1,000 ppm as nickel our carbonate.

O'Dell *et al*. (1971) study that feed intake and rate of gain were greatly reduced at a dietary Ni level of 1,000 ppm. Tissue Ni content did not differ statistically among the first 3 treatments but at 1,000 ppm Ni, a highly significant (P<.01) increase in Ni content was found in many tissues.

Kirchgessner *et al.* (1977) found that Ni supplementation at 125-, 250-, or 375-ppm levels had a non-significant ($p < 0.05$) effect whereas, at 500-level ppm depressed feed intake and growth performance in the pig.

Spear *et al.* (1984) conducted an experiment is done in the rats to know the nickel supplementation in ruminant diets has improved growth performance and feed conversion efficiency

Adams *et al.* (1992) found that increased the feed intake of sheep within 24 h. A single treatment with the longer-acting compound dexamethasone trimethyl acetate had a slower effect, but resulted in improved feed intake and enhanced body weight gain over a

period of 7 to 14 days. Treatment was effective in sheep housed individually and in sheep penned in groups

Samal and Mishra, (2011) conduct that nickel supplementation in the rats of 1000 and 2500 mg/kg of diet, but there shall indications that decreases food consumption might explain the decreased body weight gains, particularly at 2500 mg/kg of diet.

Samal and Mishra (2011) in another study, they investigate to determine the nickel in the dogs @ of 100, 1000, or 2500 mg of nickel per kg of diet (equivalent to 0, 2.5, 25, and 62.5 mg/kg of body weight per day) for a period of 2 years. In the 2500 mg/kg of diet group, decreased weight gain and food consumption shall observe.

Curlej *et al.* (2012) nickel presence at a concentration of 35 g/100 kg of rabbit feed after 3 months of systematic feeding had a negative effect on rabbit chromosomes according to the detected aneuploidy occurrence effect of nickel added at various concentrations into commercial rabbit diet during 3 months of permanent feeding in an ad libitum system.

Javed, (2013) in this study, they investigate that the influence of nickel dietary in the fish shows significantly feed conversion efficiency and condition factor shall significantly better due to dietary treatments.

Singh *et al.* (2019) conducted a study on 18 growing cattle and supplemented with Nickel (Ni) @ 1.5 mg(Ni1.5) and 3.0 mg (Ni3.0) as nickel sulfate hexahydrate/kg dry matter (DM) and was reported that linear increase ($p < 0.05$) in mean DMI and ADG without affecting feed efficiency.

2.3 Impact of nickel supplementation on antioxidant activity

Antioxidants are used as additives to prolong the shelf life of animal feeds, premixes and fats, based on their effect to prevent lipid peroxidation and oxidative rancidity during their production, processing and storage.

2.3.1. Catalase (CAT) and superoxide dismutase (SOD)

Catalase combines rapidly with H2O2 or alkyl hydroperoxides. The rate constant for the reaction catalase + H2O2 is of the order of 107 s−1 X mole−1. With the alkyl hydro-peroxides, the constant decreases with increasing chain length. In comparison to the formation of the primary compound, the back reaction can be disregarded. In this method, the enzyme activity is measured by the rate at which a filter paper disk soaked in the sample solution is carried to the surface of an H2O2 solution by the oxygen liberated. To determine the relationship between the levels of total natural scavengers of the body, the place of superoxide dismutase in this capacity, and its relation with lipid per oxidation, malondialdehyde, superoxide dismutase levels and total antioxidant status shall measure in plasma.

Misra *et al.* (1991) observed that mice 3–48 h after a single intraperitoneal injection of 170 μmol nickel (II) acetate (NiAcet)/kg body wt. Nickel treatment-induced renal LPO was greatest in the strain that is lowest in GSH and GSH-Px, but not in CAT and SOD in mice.

Das *et al.* (2001) found that findings on the effect of low supplemental Ni levels on antioxidant status are not available. Higher Ni dose (75 mg Ni/kg/day) in rats significantly increases ($p < 0.05$) hepatic lipid peroxides and decreased the activity of SOD and CAT.

Fang *et al.* (2002) founded that result SOD, and catalase (CAT) is the important enzymes and antioxidant molecule in the antioxidant system against oxidative stress. Ni – SOD is a metalloenzyme that protects cells from oxidative damage by catalyzing the cytotoxic water and molecular oxygen.

Sidhu *et al.* (2005) found that effects of nickel treatment and protein deficiency separately and in combination were studied on rat liver antioxidant defense system enzymes like catalase and superoxide dismutase (SOD). The investigations revealed a significant increase in the activity of enzymes, which include catalase and SOD in nickel treatment in combination with protein deficiency.

Zawisza and Dolezych (2010) found that supplementary of nickel influences AChE, GST and catalase activity in the body wall (increase up to 66%). Nickel pre-treatment affects the susceptibility to pesticide, which is manifested in a lower activity of GST and catalase in the fat body (from 26 to 36%) when compared with the other experimental groups.

Zawisza *et al.* (2010) conducted a study to know the impact of nickel pre-treatment augmented the response to a single diazinon application. Nickel decreased CAT activity and, in the lower concentration, inhibited intestinal absorption of glucose. AChE activity was greatly reduced compared with nickel-untreated snails. The reduction in CAT activity was similar in both groups.

Farid *et al.* (2012) investigated the short-term effects of nickel intoxication on rats' liver antioxidant defense system. After 4 weeks of oral treatment 180 mg nickel (Ni) /L and their combination. The antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) activities were decreased significantly.

Terpilowska and Siwicki (2019) present study show nickel Antioxidant activity of superoxide dismutase and catalase increased in low concentration of chromium however, they decreased in mice higher concentrations. The activity of glutathione peroxidase decreased in dose-dependent manner in all used microelements.

. **Thamizhan, P. (2020)** found that the values of total antioxidant status, the activity of SOD and GPx remained similar; however, catalase activity was higher in Murrah buffalo calves supplemented with 10 ppm Ni as compared to 5 and 0 ppm supplemented groups.

Kong *et al.* (2019) found that supplementary of nickel nanoparticles have increase CAT in adult male rats. The activity of antioxidative enzymes in rat testicular tissue is decreased by nickel nanoparticles. Vitamin C as an antioxidant can antagonize the damage induced by nickel nanoparticles. ..

2.3.2 Total antioxidant activity

Total antioxidant capacity (TAC) is the measure of the number of free radicals scavenged by a test solution being used to evaluate the antioxidant capacity of biological samples.

Anke *et al.* (1977) found that feeding chicks a diet containing 2 to 15 ppb Ni for up to 4 weeks resulted in the increased gain suggest that the higher urease activity in the Ni supplemented animals influence the recycling of nitrogen to the rumen.

Spears and hatfield (1980) These finding suggested that Ni may function, when dietary nitrogen(N) intake is low, to enhance the recycling of N to the rumen by increase ruminal epithelium antioxidant activity.

Starnes *et al.* (1982) Nickel has been found to affect oxidant activity. In lambs fed a basal purified diet low in Ni (60 ppb) and the basal diet supplemented with 5 ppm, it was found that oxidant activity was much lower in lambs fed the low Ni diet. The addition of 5 ppm of supplemental Ni greatly increased rumen bacterial oxidant activity.

Spears *et al*. (1984) study that Dietary nickel did not affect animal gain, liver cholesterol, serum protein concentrations or bacterial urease activity in the gastrointestinal tract. The addition of 5 ppm nickel to the basal dry diet reduced ammonia concentrations in the cecum.

Milne *et al.* (1990) the addition of Ni at a level of 5 mg/d given as NiCl2 .6H2O by continuous infusion into the rumen resulted in a significant increase in activity in sheep which were given a high-energy and low protein diet.

Liapi *et al.* (2011) conducted a study on twenty-eight male Wistar rats was to investigate the effects of short-term Ni-administration (as NiCl2, 13 mg/kg) on the adult rat whole brain total antioxidant status (TAS) and the activities of acetylcholinesterase. Ni short-term in vivo administration causes a statistically significant decrease in the rat brain TAS and an increase in AChE activity.

Al-Humadi and H. W. (2015) investigated the potential effects of the antioxidant property of L-cysteine (Cys) on the adult rat brain total antioxidant status (TAS) and acetylcholinesterase (AChE) activity Ni (13 mg/kg). The exposure to Cd in vivo causes a more statistically significant decrease in the rat brain TAS and an increase in AChE activity than the exposure to Ni.

2.3.3 Thio barbituric acid

Das and Das gupta, (1998) in the present study they influence the impact of nickel in rats, increased lipid peroxide formation and decreased levels of Antioxidant activities, as well as ascorbic acid depletion, have been found in the most active metabolic tissues of the body, namely, liver and kidney.

Chen *et al.* (1999) this study was undertaken to examine the oxidative effects of nickel (Ni) on rat blood and bone marrow. Nickel dose@ 100, 250, or 500 mmol/ kg which shows that there was an inverse association thiobarbituric acid elevated (TBA)-chromogen product with decreased GPx activity and a-tocopherol levels in bone marrow cells of NiCl2-treated rats.

Das *et al.* (2001) to the findings of the present study, higher Ni dose (0.97-75 mg/kg/day) in rats significantly increase hepatic lipid peroxides and a decrease in antioxidant enzymes activities.

Prasad *et al.* (2006) report that to know the supplementation nickel (250 μmoL Ni/kg body weight) to male Wistar rats results in an increase in the reduced renal glutathione content lipid peroxidation (LPO), H_2O_2 generation, blood urea nitrogen (BUN) and serum creatinine with a concomitant decrease in the activity.

Gopal *et al.* (2009) conduct that to determine the value of nickel supplementation in the fish and that is the total protein content, reduced glutathione, glutathione peroxidase and lipid peroxidation shall found to be decreased in the nickel chloride treated tissues and the treatment with CaNa2 EDTA + nickel chloride returned to near-normal levels.

2.4 Effect of nickel supplementation on blood metabolites of Muzzafarnagari sheep

2.4.1 Total leukocyte count

Schnegg and krichgessner (1975) reported that Ni deficiency in rats fd a diet containing 50 ppm fe resulted in decrease leukocyte count. Ni interacts of influences the metabolism of a number of other elements.

Spears *et al*. (1978) two experiments were conducted to study the interrelationship between nickels in rats. Rats fed the low zinc diets had decreased total leukocyte numbers in both experiments. Nickel, when supplemented with the low zinc diets, was effective in increasing leukocyte counts.

Press *et al.* (1992) reported in the Enzyme and immune histochemical methods were used to characterize the leukocyte populations present in the ileal Peyer's patches of sheep fetuses between 68 and 135 d of gestation and particularly in the period around 100 d of gestation when active lymphopoiesis begins. IgM+ and CD5+ cells were responsible for the vast majority of the increase in cell numbers.

Hujanen *et al*. (1995) they observed the exposure of nickel ions induced an orientation reaction in leukocytes similar to the polarization reaction induced by a potent peptide chemoattractant, *N*-formyl methionyl leucyl phenylalanine (fMLP), in these cells.

Pereira *et al.* (2008) The aim of this study thirty-five Wister rats were randomly distributed into three groups A, B, and C. there were significant differences between the number of leukocytes for the nickel-implanted animals and the nickel-free and control

groups after 14 days of implantation ($P < .05$). However, there was no significant difference among groups A1, A2, and A3 for the differential number of leukocytes and

for the IgA quantification, except for the number of monocytes, which was three times higher in the nickel group (A3).

Sakhare *et al*. (2019) conducted a study on four years old female sheep was presented with a history of snakebite, frothy salivation, incoordination, sternal recumbency and swelling at the right forelimb. The hematological parameters nickel showed reduced values of increased total leukocyte count.

2.4.2 Neutrophils

Neutrophils are the most common white blood cell in dogs and cats. They are the body's ‒first responders,‖ quickly arriving at the scene of an injury or infection.

Benson *et al.* (1989) study aims the Nickel sub sulfide (Ni3S2), nickel sulfate (NiSO4), and nickel oxide are encountered occupationally in the nickel refining and electroplating industries, with inhalation being a common route of exposure. All compounds produced an increase in BG, TP and an influx of neutrophils indicating the presence of a cytotoxic and inflammatory response in the lungs of exposed rats and mice.

O *et al.* (2008) observed that nano-size metal particles such as nickel (Nano-Ni), cobalt (Nano-Co), and titanium dioxide (Nano-TiO2) have much more toxic effects on rat lungs than standard-size Ni, Co, and TiO2 particles. the results showed dose-related increases of TNF-α, MIP-2, and nitrite levels in the supernatants of neutrophils treated with various doses of Nano-Ni and Nano-Co. Neutrophils treated with Nano-Ni and Nano-Co released significantly higher levels of TNF-α, MIP-2, and nitrite than those treated with Nano-TiO2 and the control

Nishi *et al.* (2009) observed that Male Wistar rats received intratracheal instillation of nickel oxide nanoparticles at 0.1 mg (0.33 mg/kg) or 0.2 mg (0.66 mg/kg),

and were dissected 3 days, 1 week, 1 month, 3 months, and 6 months after the installation. The total cell and neutrophil counts in BALF were increased from day 3 to 3 months. In lung tissue, infiltration of main neutrophils and alveolar macrophages was observed from day 3 to 6 months in alveoli.

Osman *et al.* (2012) observed that many heavy metals including chromium and nickel are widely distributed evolving occupational and environmental exposure risks which may result in adverse health nickel groups, and a pronounced increase was observed in the nickel group. Neutrophils were increased non-significantly in the nickel group.

Morimoto *et al*. (2014) reported that the micron-sized nickel oxide nanoparticle agglomerates induced neutrophil infiltration and the gene expression of the cytokine-induced neutrophil chemoattractant (CINC)-2αβ in a rat lung. In this study, we examined the expression of the CINCs family in the lung using the same rat model exposed to micron-sized nickel oxide nanoparticle agglomerates.

2.4.3 Lymphocyte

A type of immune cell that is made in the bone marrow and is found in the blood and in lymph tissue. The two main types of lymphocytes are B lymphocytes and T lymphocytes. B lymphocytes make antibodies, and T lymphocytes help kill tumor cells and help control immune responses. A lymphocyte is a type of white blood cell (NCI's Dictionary of Cancer Terms Widget). Lymphocyte count is an increase in white blood cells called lymphocytes. Lymphocytes are an important part of the immune system. They help fight off diseases, so it's normal to see a temporary rise in the number of lymphocytes after an infection.

Hernandez *et al.* (1991) nickel is a common environmental toxicant that alters the immune response. The effect of either metal, when noticeable, results in an adherence enhancement which is higher, at 10 min in lymphocytes, in male peritoneal cells and when exerted by Ni.

Zalkind *et al.* (1998) present study aimed to determine the effect of nickel-containing alloys on lymphocyte subsets in an experimental setting. One month after implantation, the mean fluorescence intensity of CD4, CD8 or Smig, in the peripheral blood lymphocytes (PBL) of the nickel alloy-implanted animals, was significantly higher than that before this procedure.

Cederbrant *et al.* (2003) lymphocytes from Ni-allergic individuals challenged with a high and a low concentration of Ni showed significantly higher cell proliferation than lymphocytes from no allergic individuals, but all subjects showed a positive LTT result.

Wu B *et al.* (2015) reported in the abnormal expression of these cytokines impacts the intestinal mucosal immune function by the pathways of reducing of lymphocyte population and activation. Also, this study first proved that $NiCl_2$ at higher levels has toxicological effects on intestinal mucosal immunity.

Yin *et al*. (2016) conducted that Two hundred and eighty-one-day-old broilers were randomly divided into four groups and fed on a control diet and three experimental diets supplemented with 300, 600, and 900 mg/kg of NiCl2 for 42 days. Lesions were observed in the NiCl2-treated groups.

Yadav *et al*. (2019) This study was aimed at evaluating the possible effects of nickel nitrate exposure on blood parameters targeting to lymphocytes, Predetermined

doses of nickel in acute (1 day) and sub-acute (7, 14, 21, 28 days) treatments revealed significant alterations in lymphocytes. The results indicate the extent of toxicity and alterations in lymphocytes under toxic stress of nickel nitrate in the albino rat.

2.4.4 Hemoglobin

Hemoglobin, also spelled hemoglobin, iron-containing protein in the blood of many animals—in the red blood cells (erythrocytes) of vertebrates that transport oxygen to the tissues. Hemoglobin forms an unstable reversible bond with oxygen.

The most common test is the packed cell volume (PCV) or hematocrit (HCT). These tests are often performed as part of a complete blood cell count (CBC). In a normal dog, 35% to 55% of the blood will be red blood cells. If the PCV is below 35%, the dog is generally considered anemic.

Jasmin and Solymoss (1975) observed that in rats, injection of nickel sulfide (5 mg) into each pole of one kidney, unlike intramuscular administration, elicits a plethoric condition a few weeks later. The resulting hematologic changes (increased hematocrit, hemoglobin, erythrocytes and circulating erythrocyte mass with normal plasma volume) indicate that the plethoric condition is due to polycythemia, which is not associated with alterations in the 2, 3-diphosphate glyceric acid content of erythrocyte.

Schnegg *et al.* (1975) observed that the essentiality of nickel could be shown by reduced growth in response to a diet with 15 ppb nickel. The F1 generation of the Ni-deficient animals, the erythrocyte count had fallen by 36%, the hematocrit by 37% and the Hb content by 44%. In the F2 generation in which the animals were given 100 ppm iron, surpassing their requirement three times, the blood parameters of the deficient animals were reduced by 8-10.

Schnegg and krichgessner (1975) suggested that Ni deficient diet fed to rats showed a decrease in RBC counts, hemoglobin. It was due to reduced Fe absorption. Ni deficiency in rats fd a diet containing 50 ppm fe resulted in decreased hemoglobin. Ni interacts of influence the metabolism of a number of other elements.

Hopfer *et al.* (1978) conducted a study on Five nickel compounds that were administered to rats by intrarenal injection to assess the relative efficacies of the compounds for induction of erythrocytosis, based upon measurements of blood packed cell volume (PCV) at intervals from one to six months after the injection.

Nielsen (1980) found that dietary nickel on hematocrit and hemoglobin level was less obvious and on plasma cholesterol was significant when rats were fed ferric-ferrous sulfate. Hematocrits and hemoglobin levels were lower in nickel-deprived than in nickel-supplemented rats (10 ng/g) only when iron was supplemented at low levels as ferric sulfate.

Parthipan *et al.* (2013) show that the level of hemoglobin, red blood cells were significantly decreased and simultaneously the white blood cells, mean cell hemoglobin and mean cell hemoglobin concentration was significantly decreased due to nickel exposure.

Al-fatlawi *et al*. (2015) showed that the level of hemoglobin, red blood cells were significantly decreased and simultaneously the white blood cells, mean cell volume, mean cell hemoglobin and mean cell hemoglobin concentration were significantly decreased due to nickel exposure.

Dahdouh *et al.* (2016) reported that Ni caused a significant decrease in body weight, food and water consumption along with significant increase in the absolute and

relative kidney weights. Hemoglobin, red blood cells count (RBC), hemoglobin (Hb) concentration, platelet counts (Plt) and packed cell volume (PCV) were significantly diminished, while white blood cells count (WBC) increased in nickel exposed mice.

Kumari *et al.* (2017) observed that hematological of *Cyprinus carpio* in the present work include the erythrocyte count, hemoglobin concentration and hematocrit value of blood. The major effects of nickel on the blood parameters are concerned with PCV total count of RBC, their size and differential count of WBC.

2.4.5 Total Immunoglobulin

Globulins are a group of proteins in your blood. They are made in your liver by your immune system. Globulins play an important role in liver function, blood clotting and fighting infection. There are four main types of globulins.

Tardivel *et al.* (1965) study aim the powdered nickel was given by mouth daily to 2 groups of 10 rabbits in amounts of 5-20 or 100-500 mg/kg. For 9 months. The serum electrophoretic pattern altered considerably, the changes occurring consistently and exclusively in the globulins. The α-1 globulins were significantly increased; the α-2 and y-globulins were reduced to 50-75% of normal; changes in β-globulins could not be statistically evaluated. A tracing is reproduced with one from a normal rabbit.

Obone *et al*. (1999) observed that Adult male Sprague-Dawley rats were given 0, 0.02, 0.05, and 0.1% nickel sulfate (NiSO4 6H2O) or 0, 44.7, 111.75, and 223.5 mg Ni/L, respectively, in their drinking water for 13 wk. Total plasma proteins, plasma albumin and globulins, and plasma glutamic pyruvic transaminase activity were all significantly decreased in 0.1% nickel sulfate-treated rats.

Hashem *et al.* (2019) this study was carried out on sixty male albino rats with average body weight (120 ± 10 g), which were divided into 6 equal groups. Gp.(1): were kept as the control on distilled water, rats in Gp. (2) received $NiCl_2$ (0.75 mg/kg BW), Gp. (3) received *A. sinensis* root extract (300 mg/kg BW) and Gp. (4) received *O. majorana* oil (0.5 ml/kg BW). $NiCl_2$ intoxicated rats in Gp. Results showed that $NiCl_2$ significantly increased serum alanine aminotransferase and alkaline phosphates activities.

Thamizhan (2020) found that the values of blood metabolites like plasma globulin, triglycerides, non-esterifies fatty acids, and creatinine and blood urea nitrogen were similar, however, plasma glucose concentration was the highest and that of total cholesterol lowest in Murrah buffalo calves supplemented with 10 ppm Ni in comparison to 5 ppm and 0 ppm supplemented groups.

2.4.6 Glucose

Glucose is the unit from which starch, cellulose and glycogen are made up and because of its special role in biological processes; there are probably more glucose groups in Nature than any other organic group. It is extremely important as one of the main energy sources for living organisms, both in plants and animals

Clary, J. J. (1975) this study was undertaken to explore the toxic effects of nickel chloride (NiCl2) on body metabolism and to elucidate the mechanism of action involved and observed A single intraperitoneal or intratracheal injection of Ni to rats caused a rapid transient increase in serum glucose, but a decrease in serum insulin.

Kirchgessner, M. and Schnegg, A. (1976) conducted that biochemical criteria on the essentiality of nickel, the activities of two dehydrogenases, malate dehydrogenase

and glucose-6-phosphate dehydrogenase, were measured in liver homogenates from two generations of rats at 30 and 50 days of age.

Horak *et al.* (1978) conduced that hyperglycemia-induced by NiCl2 and Ni (CO) 4 was not associated with inhibition of erythrocyte glycolysis measured in vitro by erythrocyte uptake of 1-14C-glucose and release of 14CO2. These findings indicate that Ni-induced hyperglycemia may be mediated by the increased pancreatic release of glucagon, but that Ni stimulation of glucagon release differs from stimulation of glucagon release by arginine or epinephrine since the Ni effect is not antagonized by somatostatin.

Foulkes, E. C. and Blanck, S. (1984) the aim of this dose of Ni reduced the calculated maximum tubular transport rate for aspartate (*T*m) and the apparent affinity constant (*K*M) by over 50% but exerted no effect on either *T*m or *K*M of cycloleucine or glucose reabsorption.

Peligero *et al.* (1985) they observed that the hyperglycemic response to nickel of female rats was more marked than that of males, with an increase in intracellular glucose, more marked during pregnancy, which even surpassed the plasma concentration of glucose.

Mas *et al.* (1986) convened that to deem the value of nickel induced considerable increases in both glucose and glucagon levels, delayed in 19-day pregnant rats with respect to controls and deep permanent decreases in glycogen and amino acids in the pregnant rat.

2.4.7 Non-esterified fatty acid (NEFA)

Non-esterified fatty acids (NEFA, or free fatty acids) are an important metabolic fuel Fatty acids released by LPL may either be sequestered in the adipocytes by esterification or released as NEFA into the plasma.

Singh *et al. (*2016) observed that NEFA reflects body fat mobilization in response to negative energy balance or stress conditions. In the present study, dietary Ni supplementation did not exert any effect on mean plasma NEFA concentration and levels were reported within the physiological limit.

2.3.8 Cholesterol and HDL-cholesterol

Das and Das Gupta, (1998) detonation that the consequence of nickel sulphate supplementation in the rat. Expression that declines in cholesterol levels in the rats.

Spears *et al.* (1984) report the supplementary of nickel diet @ 5 or 25 ppm nickel on a dry matter basis for 21 days in the pigs. Dietary nickel did not affect animal gain, liver cholesterol, serum protein concentrations or bacterial urease activity in the gastrointestinal tract.

Das *et al.* (2001) reported that the impact of nickel induced a significant increase in total cholesterol, and triglyceride levels and a significant decrease in the serum HDL cholesterol level in comparison with the control was observed in rats treated with 2.0 mg Ni/100 g BW weight.

Das *et al.* (2006) report that to determine supplementary of nickel sulfate in the rats of lipoprotein-cholesterol (2.0 mg/100 g BW). Nickel-treats in rats show a significant increase in serum low-density role, total cholesterol, triglycerides, and a significant decrease in serum high-density lipoprotein-cholesterol.

Gupta *et al.* (2008) conduct that the supplementary of nickel sulfate and potassium dichromate treats rats shows a significant increase in serum low-density lipoprotein-cholesterol (LDL-C), very low-density lipoprotein-cholesterol (VLDL-C) and triglyceride (TG) level as well as decrease in serum high density lipoprotein-cholesterol (HDL-C) level.

Pari and Elangovan (2013) found that the supplementation of nickel in the wistar rats. Subcutaneous administration of Ni (20mg/[kg body weight /day]) for 20 days showed a significant ($P < .05$) increase in total cholesterol, very low density lipoprotein cholesterol, low- density lipoprotein cholesterol, triglycerides, free fatty acids, and phospholipids, with a significant ($P < .05$) reduction in high-density lipoprotein cholesterol in plasma.

Singh *et al.* (2019) conducted a study to influence the effect of nickel in heifer that report the cholesterol concentration showed no significant effect ($P<0.05$)of treatment and their level found similar among control, 1.5 and 3.0 ppm Ni supplemented heifers.

3.1 Geographical Location of the Farm

The experiment will be conducted at LRC 2 (Livestock Research Center) Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut. Meerut is situated at 29^0 01" latitude in the north, 77^0 45" longitudes in the East and at an elevation of 224.659 meters above mean sea level. The total geographical area of the Meerut division under western zone of U.P. is 20624 Km^2. In summer, the highest temperature rises up to 45° C and in winter there is a remarkable fall of temperature sometimes up to freezing point.

3.2 Design of experiment, feeding and management

Eighteen Muzzafarnagari sheep (8-12-month-old) will be selected from Livestock Research Center 2, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut and randomly divided into three groups (n = 7) on body weight and age basis. The Muzzafarnagari sheep will be fed a total mixed ration (TMR) containing concentrate, green fodder, and straw in the ratio of 45:35:20 to meet their nutrient requirement as per the recommendation of NRC (2001). The supplementation of nickel doses will be given to the animals as followings-

Number of animals	**Group**	**Diet**	**Supplementation of Nickel**
21 Muzzafarnagari sheep	Control (7)	Basal	No supplementation
	T_1 (7)	Basal	1.5mg/kg DM/day
	T_2 (7)	Basal	3.0mg/kg DM/day

The calculated doses of nickel were mixed in a small amount of concentrate and fed individually to each animal for 90 days of study period. Clean and fresh tap water will be offered *ad-lib*. Experimental animals will be kept under conventional housing system. The shed will be washed and cleaned daily to prevent the chances of any infections. During the entire period of study, various management practices viz., deworming, washing, grooming and treatment, etc. will be followed as per the standard procedure of Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut. The duration of experiment will be 90 days.

3.3 Growth performance

3.3.1 Live body weight (BW) and body weight gain (BWG)

BW will be measured by digital electronic balance of all Muzzafarnagari sheep before start of experiment and repeated at fortnightly intervals for 90 days of experiment period. BWG will be calculated as-

BWG = BW of current fortnight -BW of previous fortnight

3.3.2 Feed consumption (FC) and feed conversion ratio (FCR)

FC will be calculated as subtraction of residual feed from offered feed per day at fortnightly intervals. The FCR will be calculated as per formula-

FCR = FC (g) / BWG (g)

3.4 Blood collection

Blood samples will be collected from jugular vein of Muzzafarnagari sheep on fortnightly interval in the EDTA coated Vacationer tube at 07.00 a.m. before feeding and watering. Fraction of blood will be used in the estimation of hemoglobin, total leukocyte counts, lymphocyte and neutrophil. Rest of blood sample will be centrifuged at 3000 rpm for 30 min for separating plasma. The plasma will be stored at -20 ^{o}C till further analysis of total immunoglobulin, cholesterol, HDL-cholesterol, thiobarbituric acid reactive substances, total antioxidant activity, glucose, non-esterifies fatty acids. The hemolysate will be used in the estimation of superoxide dismutase, catalase, and glutathione peroxidase.

3.5 Antioxidant status

3.5.1 Superoxide dismutase

Reagents required

a) Pyrogallol (2mM) 25.2 mg was dissolved in 100 ml of 10 m MHCl

b) Tris buffer (50 mM)

605 mg of tris buffer was dissolved in 100 ml of distilled water. 39 mg of Diethylenetriaminepentaacetic acid was added to 100 ml of the buffer. PH adjusted to 8.2 using HCl.

The enzyme activity was assayed by the method of **Marklund and Marklund (1974).** The reaction mixture contained a different concentration of appropriately diluted lysate ranging from 0.2 of 2.0 ml which were made up to 3 ml by tris-HCl buffer (50 mM, pH 8.2) containing 1mM diethylenetriaminepentaacetic acid and 0.2 ml of 2 mMpyrogallol. A standard was prepared without sample. The pyrogallol auto oxidation rate was taken from the increase in absorbance at 420 nm against a reference cuvette containing 3.0 ml, tris buffer using Specord 200 Double Beam UV-visible Spectrophotometer. The absorbance increase was 0.02 min^{-1} in the absence of superoxide dismutase enzyme. The inhibition of pyrogallol auto-oxidation is brought about by superoxide dismutase which was employed for the determination of enzyme activity. An enzyme unit was defined as the amount of enzyme that inhibits the reaction by 50 percent.

3.5.2 Catalase

The activity of the enzyme was estimated Spectro photometrically using the method described by

Reagents:

1. Phosphate buffer (50 mM)

Dissolve

(a) 6.81 gm KH_2PO_4/L

(b) 8.90 gm $Na_2HPO_4.2H_2O$/L

Kept the solution (a) in a beaker, and then slowly added solution (b) to solution (a) in 1: 1.5 (v/v), adjusted pH to 7.0

2. H_2O_2 (30 mM)

Dilute 0.34 ml of 30 % H_2O_2 with phosphate buffer to 100 ml.

Blank	**Sample**
Phosphate buffer - 2.9 ml	1.9 ml
H2O2 -	1 ml
RBC lysate - 25-100 µl	25-100 µl

The reaction was started by adding H_2O_2. The decomposition of H_2O_2 can be shown by decrease in absorbance at 240 nm. The decrease in absorbance observed for 65 seconds and difference between 5 seconds and 65 seconds of absorbance was taken. Using an extinction coefficient of 0.0394 ml mM^{-1} cm^{-1} the enzyme activity was calculated and expressed as µ moles of H_2O_2 consumed/min/g Hb in blood.

3.5.3. Total antioxidant activity

Total antioxidant activity was measured by ferric reducing antioxidant power (FRAP) assay of **Benzie and Devaki (2018)**. FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method, employing easily reduced oxidant system present in stoichiometric excess.

Reagents

1. FRAP Reagent

Acetate buffer 3.0 mM, pH 3.6: Weighed 3.1 gm sodium acetate trihydrate and added 16 ml of glacial acetic acid and made the volume to 1.0 liter with distilled water.

a) **Ferric chloride 2 mM in 40 mM HCl.**

b) **Tripyridyl triazine 10 mM**

The working FRAP reagent was prepared by mixing A, B & C in the ratio of 10:1:1, at the time of use.

1. **Ferrous sulphate 1mM**
2. **Ascorbic acid 100 μM**

Procedure: Plasma (100 μl) was mixed with 3 ml of working FRAP reagent and absorbance was measured at 0 minute after vertexing. Thereafter, samples were placed at 37°C in water bath and absorbance was measured after 4 min. Ascorbic acid standards (100 μM-1000 μM) were processed in the same way.

Sample calculation: Results were calculated as follows.

$$\text{FRAP value of sample (μmol/L)} = \frac{A-B}{X-Y} \times 100$$

A = Reading of sample at 0 minute

B = Reading of sample at 4 minutes

X = Reading of standard at 0 minute

Y = Reading of standard at 4 minutes

100 = FRAP value of 100 μM standard

3.5.4 Estimation of Thiobarbituric acid reactive substances:

The extent of lipid peroxidation, an index of oxidative stress was measured as Thiobarbituric acid reactive substances formed. Lipid peroxides were measured by the TBA test method of Asakawa and Matsushita (1979).

Reagent preparation:

A) Glycine HCl buffer (0.2 M, pH 3.6)

1.5 g of Glycine was dissolved in distilled water. The pH was adjusted to 3.6 with HCl (1N) and volume made to 100 ml.

B) TBA reagent (0.5%)

The TBA reagent was made by dissolving 0.5 g of TBA and 0.3 g of SDS in 100 ml water.

C) Ferric chloride solution

270 mg of $FeCl_3.6H_2O$ was dissolved in 100 ml water.

D) BHT ethanol solution

220 mg of Butylatedhydroxytoluene was dissolved in 100 ml of ethanol.

Procedure:

To 0.1ml of sample, 0.1ml of ferric chloride solution, 0.1ml of BHT ethanol solution, 1.5ml of 0.2M glycine HCl buffer and 1.5ml of TBA reagent were added. The mixture was heated for 15 minutes in a boiling water bath. Then it was cooled in ice water, 1.0 ml of glacial acetic acid and 2ml of chloroform were added. The mixture was shaken and centrifuged for 10 minutes. The optical density of the supernatant was determined at 532 nm with the help of Specord 200 Double Beam UV/visible Spectrophotometer.

A reagent blank was run simultaneously. The molar extinction coefficient used to calculate the amount of malonaldehyde was 1.56×10^5 m^{-1}cm-

3.6 Immune status:

3.6.1 Total leukocyte count (TLC)

Anti coagulated blood was sucked into WBC pipette up to 0.5 mark followed by WBC diluting fluid up to 11 marks. The pipette was then rotated between the palm for the few seconds in orders to facilitate proper mixing of the contents. Following few minutes counting chamber was charged after discarding the first few drops of diluted sample. Once the cells settle down, WBCs were counted in the four large squares. This number is multiplied by 50 to calculate TLC count (10^3cells /mm^3).

3.6.2 Lymphocyte and Neutrophil

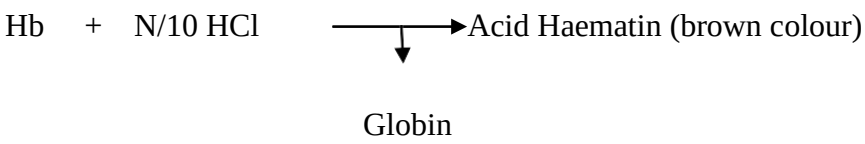
A thin blood film was made by spreading a blood drop evenly on clean grease free slide using smooth edged spreader. Modification of Romanowsky's stain (**Marshal T. H. *et al*. 1975)** namely Leishman's stain was use. For Giemsa's staining the air-dried blood smears were prefixed with acetone free methanol for 5 minutes. In general terms, 100 white blood cells should be counted and classified according to the morphologic and staining characteristic. Counting is usually carried out using a manual differential cell counter. The differential white blood cell count is expressed as a percentage of the individual cell group.

3.6.3 Hemoglobin

Sahli's acid hematin method

Procedure

Hemoglobin is converted to acid hematin by the action of HCl. The acid hematin solution is further diluted until its color matches exactly with that of the permanent standard of the comparator block. The hemoglobin concentration read directly from the calibration tube.



Procedure

1. Clean the hemoglobin meter tube and pipette and ensure that they are dry.
1. Fill the hemoglobin meter tube with N/10 HCl up to its lowest mark (10% or 2 g %) with the help of a dropper.
2. Dip the tip of the hemoglobin meter pipette into the blood and suck up to 20 cu mm mark of the pipette.
3. Wipe the tip of the pipette. Immediately transfer the 0.02 ml of blood from the pipette into the hemoglobin meter tube containing N/10 HCl by immersing the tip of the pipette in the acid solution and blowing out blood from the pipette. Rinse the pipette 2-3 times by drawing up and blowing out the acid solution.
4. Leave the solution in the hemoglobino meter tube for about 10 min.
5. After 10 min., dilute the acid haematin by adding DW drop by drop. Mix it with stirrer.
6. Match the color of the solution in the tube with standard of the comparator.
7. If the color of the test solution is darker continue dilution till it matches with that of the standard.

8. Note the reading when colour of the test solution exactly matched with standard and express the Hb content as g%.

3.6.4 Total immunoglobin

The zinc turbidity method (**Mc Ewan and Fisher, 1970**) was used to measure plasma immunoglobulins.

Reagents: $ZnSO_4$, Fetal calf serum, Rabbit gamma globulin.

Test reagent: It was prepared by taking 4.1ml of 5% $ZnSO_4$ and the volume was made to 1 liter with freshly prepared distilled water.

Procedure: Take 100 µl plasma samples in a clean dry test tube and add 12 ml of test reagent, kept at room temperature for 1 hour. The standards (12-50 mg/ml) were prepared in fetal calf serum and were processed similar to samples. OD was taken at 460 nm. Then the OD of samples was plotted against the standard curve and thus, the concentrations of total immunoglobulin in the samples were estimated and expressed as mg/ml of plasma.

3.7 Blood metabolites

3.7.1 Glucose

Glucose was estimated in plasma samples by GOD-POD method using kits from ERBA diagnostics Mannheim Germany.

Principle

Trinder's method

Glucose in the sample is oxidized to yield gluconic acid and hydrogen peroxide in the presence of Glucose oxidase. The enzyme peroxidase catalyzes the oxidative coupling of 4-aminoantipyrine with phenol to yield a colored quinonimine complex, with absorbance proportional to the concentration of glucose in sample.

Glucose + O_2 + H_2O ⟶ Gluconic acid + H_2O_2

H_2O_2 + 4 HBA +4AAP ⟶ Quinonimine Dye + $2H_2O$

4AAP: 4 amino antipyrine

4HBA: 4-Hydroxy benzoic acid

The intensity of the pink color formed is proportional to the glucose concentration and can be measured photometrically at 505nm.

Assay procedure

Pipette into tubes marked	**Blank**	**Standard**	**Test**
Working Reagent	1000 µl	1000 µl	1000 µl
Distilled water	10 µl	------	-------
Standard	------	10 µl	-------
Test	------	------	10 µl

Mixed well and incubated for 15 minutes at 37ºC. Read the absorbance of Standard and each test tube against reagent blank at 505nm (500-540) / 600nm on spectrophotometer (Thermo scientific™, USA).

Calculation

$$\text{Glucose (mg/dl)} = \frac{\text{Abs. of test}}{\text{Abs. of standard}} \times \text{Concentration of standard } (\text{mg/dl})$$

Estimation of biochemical parameters in lab of Veterinary Physiology and Biochemistry

3.7.2 NEFA

NEFA was estimated by the extraction method modified. The method is being discussed under the following headings.

Reagent preparation:

Copper reagent: A mixture of 5ml of triethanolamine and 10ml of 1M aqueous cupric nitrate [Cu $(NO_3)_2.3H_2O$] was diluted to 100ml with saturated sodium chloride solution. The pH was adjusted to 8.3 with 1N sodium hydroxide solution. The mixture was stored in the dark room temperature in order to ensure that the material remained stable for a period of at least 4-5 months.

Colour reagent: 0.5% Sodium diethyl dithiocarbamate solution in n-butanol, i.e., 0.5 gm per 100ml.

Solvent mixture: Chloroform, n-Heptane and methanol (all GR grade) were mixed in proportion of 49:49:2, respectively, and the mixture was designated as CHM.

Procedure: 0.5 ml of plasma sample was taken in a 16×125 mm screw cap test tube. Then 0.1ml of 0.7 N HCl was added to the plasma sample. The mixture was shaken on a vortex test tube mixer. Following this, 2 ml of copper reagent and 6 ml of the solvent mixture was added. The contents were shaken for 30 minutes on shaker at 240 rpm and then centrifuged for 10 minutes at 4°C at 3000 rpm in a refrigerated centrifuge. Solvent layer 3.5 ml was separated to an acid washed test tube containing 0.1 ml of the Copper reagent. The contents were mixed well, then the colour intensity was measured within 1 hr. at 440 nm using spectrophotometer against blank prepared in the same manner and using 0.5 ml double distilled water in place of plasma. The content of NEFA can be calculated from the standard curve.

Preparation of standard curve:

The standard curve was prepared with palmitic acid as specified as given below:

0.2 M solution of palmitic acid (5.12 g/100 ml) was prepared in solvent mixture as described above. One ml of this stock solution was diluted to 100ml with solvent mixture giving the final concentration of 2,000 µmol/lit 0.1 ,0.2, and 0.8ml aliquots of this solution having a concentration of 0.2,0.4,0.8,and 1.6 µmol of palmitic acid were taken and the colour was developed in the similar manner as given in procedure described above. The blank was prepared simultaneously without palmitic acid. The concentration of palmitic acid was plotted against absorbance recorded at 440 nm. The values were expressed as µmol NEFA/liter of plasma.

3.7.3 Total Cholesterol

Total Cholesterol was estimated in plasma samples by CHOD-PAP method using kits from ERBA diagnostics Mannheim Germany.

Methodology

Modified Roeschlau's Method

Principle

The estimation of cholesterol involves the following enzyme catalyzed reactions.

1. Cholesterol ester $\longrightarrow$ Cholesterol + fatty acid
2. Cholesterol + O_2 $\longrightarrow$ Cholest-4-en-3-one + H_2O_2
3. $2H_2O_2$ + 4AAP + Phenol $\longrightarrow$ $4H_2O$ + Quinonimine

Absorbance of quinonimine so formed is directly proportional to the Cholesterol concentration in the specimen.

Assay procedure

Pipette into tubes marked	**Blank**	**Standard**	**Test**
Working Reagent	1000 µl	1000 µl	1000 µl
Distilled water	20 µl	------	-------
Standard	------	20 µl	-------
Test	------	------	20 µl

Mixed well and incubated for 10 minutes at 37ºC. Aspirate blank followed by standard and tests. Read the absorbance of Standard and each test tube against reagent blank at 505nm or 505/ 670nm on spectrophotometer (Thermo scientific™, USA).

3.7.4 HDL Cholesterol

HDL Cholesterol was estimated in plasma samples by Phosphotungstic acid method using kits from ERBA diagnostics Mannheim Germany.

Methodology: -

Principle: Chylomicrons, LDL and VLDL (low and very-low-density lipoprotein) are precipitated from serum by phosphotungstate in the presence of divalent cations such as magnesium. The HDL cholesterol remains unaffected in the supernatant and is estimated using ERBA Cholesterol reagent .

Serum/plasma $\xrightarrow[Mg^{2+}]{\text{Phosphotungstate}}$ HDL + (LDL + VLDL + Chylomicrons)

(Supernatant)

Assay Procedure

Pipette into tubes marked	**Blank**	**Standard**	**Test**
Cholesterol Working Reagent	1000 µl	1000 µl	1000 µl
Distilled water	50 µl	------	-------
Standard	------	50 µl	-------
Test	------	------	50 µl

Mixed well and incubated for 10 minutes at 37ºC or 12 minutes at 30 ºC. Read the absorbance of Standard and each test tube against reagent blank at 505nm or 505/670nm on spectrophotometer (Thermo scientific™, USA).

$$\text{Calculation} = \frac{\text{Abs. of Test}}{\text{Abs. of Standard}} \times \text{Concentration of standard (mg/dl)}$$

$$\text{HDL Cholesterol} = \frac{\text{Abs. of Test}}{\text{Abs. of Standard}} \times 25$$

3.8 Statistical Analysis

All the results were expressed as mean +_ S.D. The comparison of the control with that of the treated data was statistically analysed by using student's t-test to establish the validity of the effect observed (Ipsen andFeigel, 1970).

The 'p' values are significant according to the following convention:

$p>0.05$ = non-significant (*)

$p<0.05$ = significant (**)

$p<0.001$ - highly significant (*)

The results of the current study entitled **"Effect of nickel supplementation on growth performance, antioxidant and blood metabolites of Muzaffarnagri sheep"** have been presented in this chapter.

4.1. The effect of nickel supplementation on growth performance

4.1.1. Body weight

The data of body weight (BW) of Muzaffarnagari sheep of all the three groups measured at fortnight intervals over the 90 days trial period has been given in Table 4.1and Figure 4.1.Initial BW was 30.29, 30.33 and 30.74 kg in T0, T_1 and T_2 groups, respectively. The final BW of corresponding groups was 34.03, 34.18 and 34.98 kg on 90-day of trial. The mean BW and BW on different fortnights were statistically (P>0.05) similar in all three groups.

Table 4.1. Effect of nickel supplementation on body weight (kg) of Muzaffarnagari sheep

Fortnight	Treatment			SEM	P-value		
	T0	T1	T2		Contrast	Linear	Quadratic
0	30.29	30.33	30.74	3.67	0.958	0.795	0.899
1	30.90	30.97	31.44	3.68	0.941	0.751	0.894
2	31.50	31.61	32.15	3.68	0.920	0.706	0.885
3	32.12	32.25	32.86	3.68	0.899	0.669	0.874
4	32.81	32.89	33.56	3.72	0.892	0.669	0.841
5	33.43	33.55	34.27	3.72	0.868	0.628	0.841
6	34.03	34.18	34.98	3.73	0.841	0.590	0.828
Mean	**32.15**	**32.25**	**32.86**	**3.70**	**0.516**	**0.289**	**0.658**

T0, no nickel group, T1, nickel supplemented group (1.5mg/kg DM); T2, nickel supplemented group (3 mg/kg DM); SEM, Standard error mean

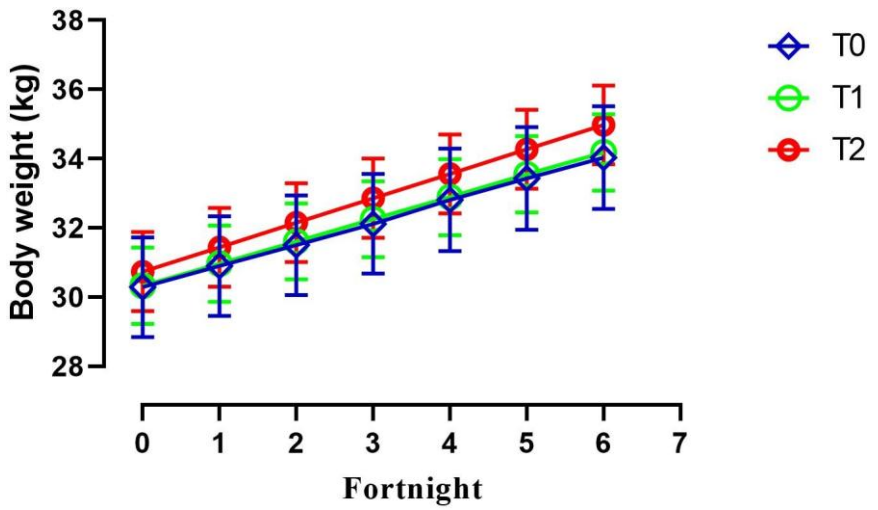


Figure 4.1 Fortnightly changes of body weight (BW) in Muzaffarnagari sheep supplemented with nickel.

4.1.2. Body weight gain

The fortnightly body weight gain (BWG) of Muzaffarnagari sheep of T0, T_1 and T_2 groups have been presented in Table 4.2.and Figure 4.2. The BWG was statistically greater in the group receiving 3.0 mg nickel/kg DM than 1.5 mg nickel/kg DM supplemented and non-supplemented groups on 1, 2, and 5 fortnights of the study period. Moreover, the mean value of BWG was also statistically ($P<0.05$) higher in the T2 group as compared to all other groups. However, there was no significant ($P>0.05$) difference observed between T0 and T1.

Table 4.2. Effect of nickel supplementation on body weight gain (kg/d) of Muzaffarnagari sheep

Fortnight	Treatment			SEM	P-value		
	T0.	T1	T2		Contrast	Linear	Quadratic
1	0.040^{a}	0.043^{b}	0.047^{c}	0.001	<0.001	<0.001	0.210
2	0.041^{a}	0.043^{b}	0.047^{c}	0.002	<0.001	<0.001	0.126
3	0.041^{a}	0.042^{a}	0.047^{b}	0.002	<0.001	<0.001	0.188
4	0.046	0.042	0.047	0.007	0.578	0.910	0.305
5	0.041^{a}	0.044^{b}	0.047^{c}	0.002	<0.001	<0.001	0.849
6	0.041^{a}	0.042^{a}	0.047^{b}	0.002	<0.001	<0.001	0.021
Mean	**0.042^{a}**	**0.043^{a}**	**0.047^{b}**	**0.002**	**<0.001**	**<0.0001**	**0.049**

T0, no nickel group, T1, nickel supplemented group (1.5mg/kg DM); T2, nickel supplemented group (3 mg/kg DM); SEM, Standard error mean

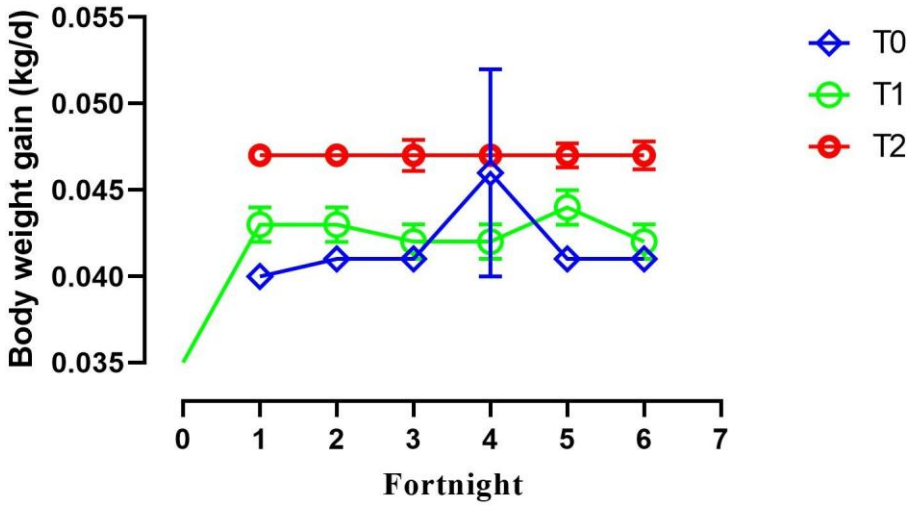


Figure 4.2 Fortnightly changes of body weight gain (BWG) in Muzaffarnagari sheep supplemented with nickel.

4.1.3. Feed consumption

Feed consumption (kg/d) in T0, T_1, and T_2 groups during different fortnights of the experimental period has been presented in Table 4.3 and Figure 4.3. Feed consumption was not affected with the supplementation of nickel and was reported statistically similar in all three groups on each fortnight of the study period.

Table 4.3. Effect of nickel supplementation on Feed consumption (kg/d) of Muzaffarnagari sheep

Fortnight	Treatment			SEM	P-value		
	T0	T1	T2		Contrast	Linear	Quadratic
1	0.56	0.56	0.57	0.06	0.946	0.748	0.949
2	0.57	0.57	0.58	0.06	0.920	0.712	0.873
3	0.58	0.58	0.59	0.06	0.913	0.716	0.833
4	0.59	0.59	0.60	0.07	0.882	0.686	0.775
5	0.60	0.60	0.62	0.07	0.882	0.653	0.835
6	0.61	0.62	0.63	0.07	0.840	0.587	0.834
Mean	**0.58**	**0.59**	**0.60**	**0.06**	**0.539**	**0.312**	**0.649**

T0, no nickel group, T1, nickel supplemented group (1.5mg/kg DM); T2, nickel supplemented group (3 mg/kg DM); SEM, Standard error mean

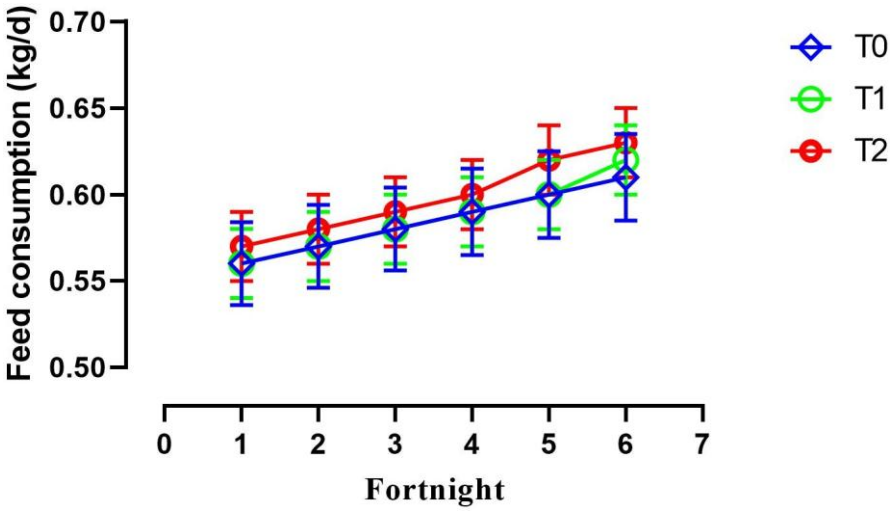


Figure 4.3 Fortnightly changes of Feed consumption in Muzaffarnagari sheep supplemented with nickel.

4.1.4. Feed conversion ratio

The feed conversion ratio (FCR) of experimental animals at different fortnight is given in Table 4.4 and Figure 4.4.The FCR was not varied significantly among the groups on 2, 3, 4, 5, and 6 fortnights of the trial period. The mean FCR was statistically lower in the T2 group as compared to T0 and T1 groups.

Table 4.4 Effect of nickel supplementation on Feed conversion ratio of Muzaffarnagari sheep

Fortnight	Treatment			SEM	P-value		
	T0	T1	T2		Contrast	Linear	Quadratic
1	13.83^{b}	12.99^{ab}	12.08^{a}	1.38	0.052	0.016	0.947
2	13.97	13.31	12.21	1.62	0.096	0.034	0.745
3	14.14	13.70	12.65	1.51	0.130	0.052	0.636
4	13.41	14.00	12.82	2.06	0.538	0.577	0.340
5	14.80	13.71	13.06	1.72	0.132	0.049	0.759
6	15.09	14.62	13.39	1.42	0.056	0.021	0.526
Mean	**14.21^{b}**	**13.72^{b}**	**12.70^{a}**	**1.62**	**<0.001**	**<0.001**	**0.345**

T0, no nickel group, T1, nickel supplemented group (1.5mg/kg DM); T2, nickel supplemented group (3 mg/kg DM); SEM, Standard error mean

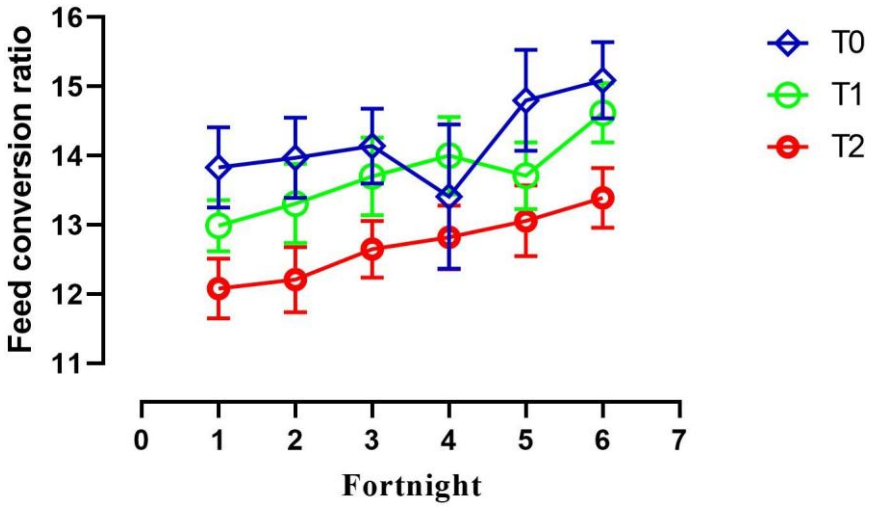


Figure 4.4 Fortnightly changes of feed conversion ratio in Muzaffarnagari sheep supplemented with nickel.

4.2. Effect of nickel supplementation on antioxidant status

4.2.1. Superoxide dismutase

The data of superoxide dismutase (SOD) activity of all experimental groups is presented in Table 4.5.and Figure 4.5. Plasma concentration of SOD the starting of the experiment was 2.14, 2.16, and 2.18 U/mg Hb in T0, T_1, and T_2 groups, respectively and

the corresponding values at the end of the experiment were 2.22, 2.07, and 2.04U/mg Hb. The SOD activity was not affected with the supplemental nickel and remained similar statistically (P>0.05) in all the groups on all the fortnights of the study period. Likewise, the mean activity of SOD was also not varied significantly (P>0.05) among the groups.

Table 4.5. Effect of nickel supplementation on SOD (U/mg Hb) of Muzaffarnagari sheep

Fortnight	Treatment			SEM	P-value		
	T0	T1	T2		Contrast	Linear	Quadratic
0	2.14	2.16	2.18	0.56	0.986	0.869	0.978
1	2.16	2.19	2.13	0.66	0.982	0.926	0.872
2	2.20	2.22	2.19	0.40	0.985	0.953	0.873
3	2.18	2.20	2.26	0.47	0.943	0.744	0.929
4	2.21	2.23	2.06	0.51	0.788	0.577	0.693
5	2.20	2.15	2.28	0.37	0.764	0.66	0.560
6	2.22	2.07	2.04	0.46	0.700	0.438	0.756
Mean	**2.15**	**2.17**	**2.16**	**0.52**	**0.963**	**0.784**	**0.993**

T0, no nickel group, T1, nickel supplemented group (1.5 mg/kg DM); T2, nickel supplemented group (3 mg/kg DM); SEM, Standard error mean

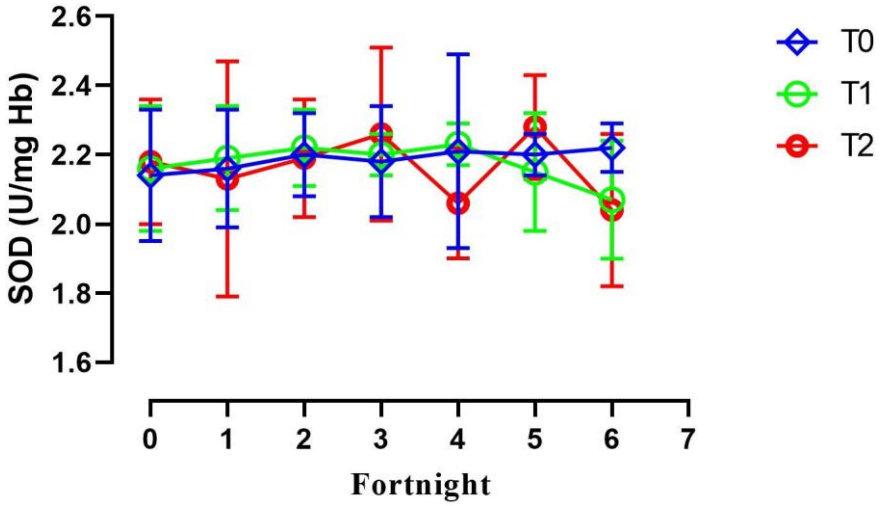


Figure 4.5 Fortnightly changes of superoxide dismutase in Muzaffarnagari sheep supplemented with nickel.

4.2.2. Catalase

The catalase activity of all the groups is presented in Table 4.6 and Figure 4.6. Catalase concentrations at of experiment were 0.31, 0.29 and 0.26 moles of H_2O_2 consumed/min/mg Hb in T0, T_1 and T_2 groups respectively. in the 0 fortnight and the corresponding values at the six-fortnight were 0.37, 0.40 and 0.29 mole of H_2O_2 consumed/min/mg Hb. Catalase activity was not varied significantly among groups on 0, 1 and 3 fortnights. However, the mean catalase activity was shown a statistical difference and was observed significantly ($P<0.05$) lower in T2 as compared to control and T1 groups.

Table 4.6 Effect of nickel supplementation on Catalase (µmol H2O2 consumed/min/g Hb) of Muzaffarnagari sheep

Fortnight	Treatment			SEM	P-value		
	T0	T1	T2		Contrast	Linear	Quadratic
0	0.31	0.29	0.28	0.03	0.326	0.480	0.924
1	0.34	0.32	0.28	0.05	0.056	0.022	0.509
2	0.35^{b}	0.34^{b}	0.27^{a}	0.03	<0.001	<0.001	0.011
3	0.36	0.35	0.30	0.07	0.270	0.128	0.607
4	0.37^{b}	0.36^{b}	0.31^{a}	0.04	0.003	0.002	0.156
5	0.36^{b}	0.35^{b}	0.30^{a}	0.03	0.001	0.001	0.046
6	0.37^{a}	0.40^{ab}	0.29^{b}	0.05	0.002	0.007	0.006
Mean	**0.35^{b}**	**0.34^{b}**	**0.29^{a}**	**0.04**	**<0.001**	**<0.001**	**0.001**

T0, no nickel group, T1, nickel supplemented group (1.5mg/kg DM); T2, nickel supplemented group (3 mg/kg DM); SEM, Standard error mean

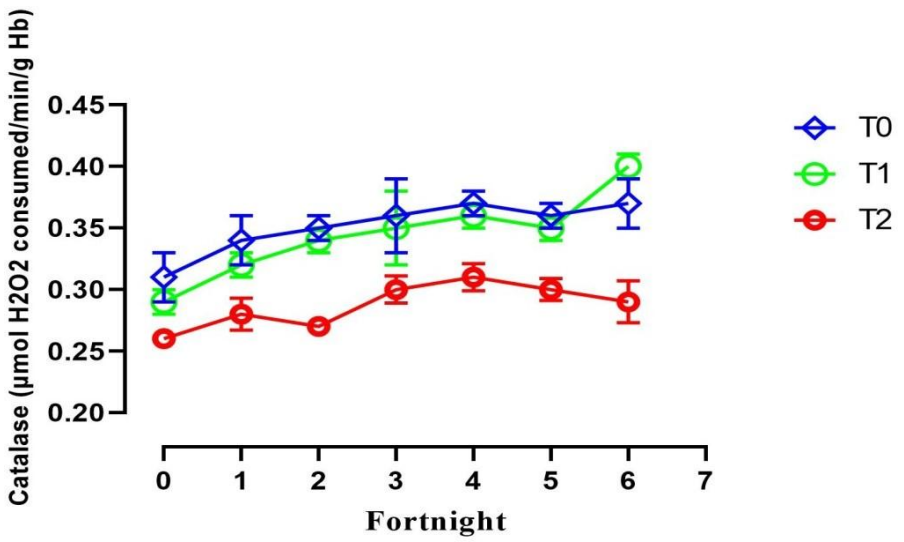


Figure 4.6 Fortnightly changes of catalase in Muzaffarnagari sheep supplemented with nickel.

4.2.3. Total antioxidant activity

The influence of nickel supplementation on the total antioxidant activity of sheep is presented in Table 4.7. and Figure 4.7. Total antioxidant activity was not affected by nickel supplementation and was reported statistically (P>0.05) similar in all three groups on entire fortnights of the study. Moreover, the mean activity of catalase was also not varied statistically (P>0.05) in the groups.

Table 4.7. Effect of nickel supplementation on total antioxidant activity (µmol/ml) of Muzaffarnagari sheep

Fortnight	Treatment			SEM	P-value		
	T0	T1	T2		Contrast	Linear	Quadratic
0	1.25	1.27	1.30	0.05	0.242	0.098	0.873
1	1.27	1.30	1.33	0.08	0.411	0.191	0.889
2	1.29	1.33	1.35	0.13	0.645	0.369	0.828
3	1.32	1.31	1.34	0.11	0.875	0.743	0.695
4	1.35	1.34	1.37	0.12	0.888	0.758	0.712
5	1.34	1.36	1.31	0.13	0.806	0.671	0.624
6	1.33	1.35	1.33	0.15	0.929	0.940	0.711
Mean	**1.31**	**1.32**	**1.33**	**0.11**	**0.538**	**0.275**	**0.828**

T0, no nickel group, T1, nickel supplemented group (1.5mg/kg DM); T2, nickel supplemented group (3 mg/kg DM); SEM, Standard error mean

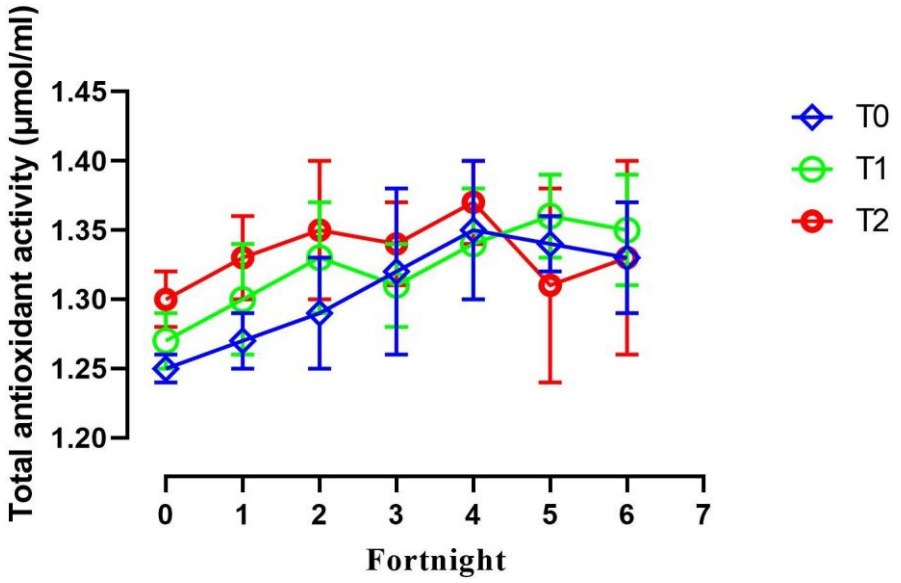


Figure 4.7 Fortnightly changes of total antioxidant activity in Muzaffarnagari sheep supplemented with nickel.

4.2.4. Thiobarbituric acid reactive substance

The data of thiobarbituric acid reactive substance of all three groups is presented in Table 4.8 and Figure 4.8.The mean value of thiobarbituric acid was1.38, 1.35 and 1.33 nmol/ml in the control, T_1 and T_2 groups, respectively, and the observed value was statistically similar in the groups. The TBARS levels on 0, 1, 2, 3, 4, 5, and 6 fortnight of the study were statistically ($P>0.05$) similar in all three groups.

Table 4.8. Effect of nickel supplementation on Thiobarbituric acid reactive substance (nmol/ml) of Muzaffarnagari sheep

Fortnight	Treatment			SEM	P-value		
	Cont.	T1	T2		Contrast	Linear	Quadratic
0	1.38	1.35	1.33	0.06	0.311	0.138	0.782
1	1.39	1.39	1.39	0.14	0.999	0.984	0.972
2	1.42	1.40	1.35	0.08	0.297	0.133	0.729
3	1.47	1.38	1.36	0.10	0.086	0.035	0.509
4	1.44	1.44	1.45	0.15	0.977	0.833	0.968
5	1.41	1.42	1.40	0.14	0.958	0.919	0.788
6	1.40	1.41	1.39	0.16	0.981	0.903	0.880
Mean	**1.41**	**1.40**	**1.38**	**0.12**	**0.383**	**0.167**	**1.000**

T0, no nickel group, T1, nickel supplemented group (1.5 mg/kg DM); T2, nickel supplemented group (3 mg/kg DM); SEM, Standard error mean

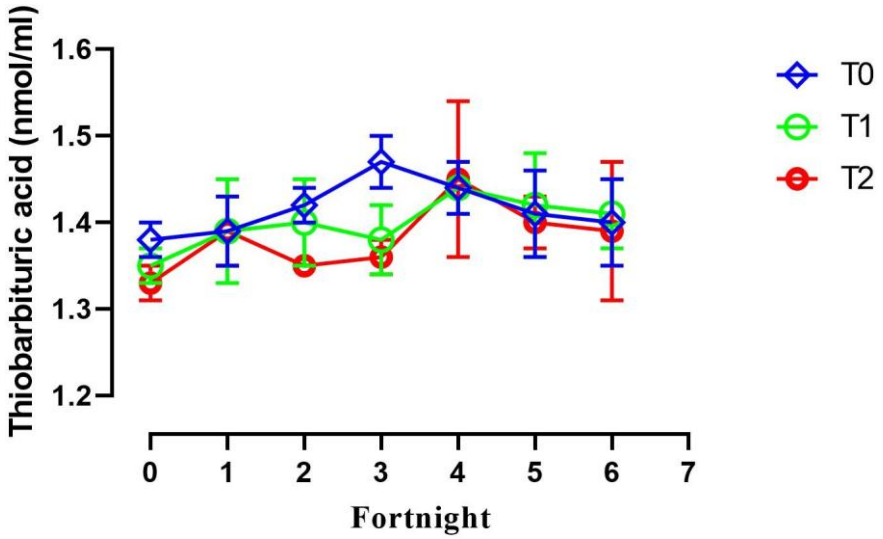


Figure 4.8 Fortnightly changes of thiobarbituric acid in Muzaffarnagari sheep supplemented with nickel.

4.3. Effect of nickel supplementation on immune status

4.3.1. Total leukocyte count

The total leukocyte count (TLC) of a different group of Muzaffarnagari sheep has been depicted in table 4.9 and figure 4.9.There was no significant difference was observed in the overall mean of TLC of all three groups and the value was 7.96, 7.98 and 7.97($\times 10^3$/µl) in T0, T1 and T2 groups, respectively. Additionally, TLC did not vary statistically (P>0.05) among the group during different fortnights of the study period.

Table 4.9. Effect of nickel supplementation on TLC ($\times 10^3$/µl) of Muzaffarnagari sheep

Fortnight	Treatment			SEM	P-value		
	Cont.	T1	T2		Contrast	Linear	Quadratic
0	7.79	7.82	7.85	0.61	0.981	0.849	0.984
1	8.51	8.13	8.35	0.65	0.490	0.611	0.285
2	7.96	7.98	7.69	0.67	0.609	0.411	0.583
3	8.61	8.09	8.13	0.69	0.233	0.158	0.334
4	7.76	7.58	7.66	0.69	0.867	0.710	0.661
5	8.44	8.46	8.18	0.66	0.635	0.433	0.599
6	7.95	7.82	7.96	0.52	0.835	0.996	0.554
Mean	**7.96**	**7.98**	**7.97**	**0.65**	**0.295**	**0.164**	**0.478**

T0, no nickel group, T1, nickel supplemented group (1.5 mg/kg DM); T2, nickel supplemented group (3 mg/kg DM); SEM, Standard error mean

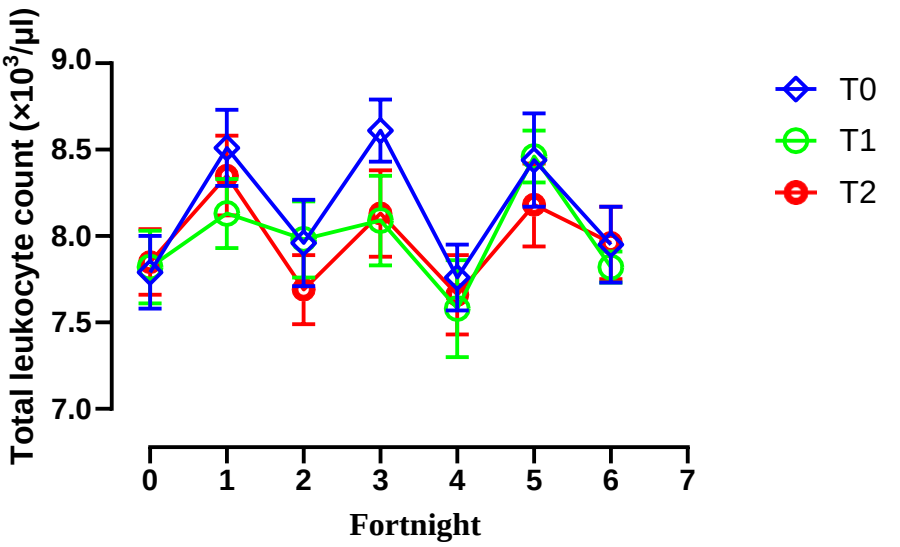


Figure 4.9 Fortnightly changes of total leukocyte count in Muzaffarnagari sheep supplemented with nickel.

4.3.2. Neutrophil

The impact of nickel supplementation on neutrophil concentration of Muzaffarnagari sheep has been shown in Table 4.10 and outlined in Figure 4.10. On the 0-day of the experiment, the concentration of neutrophil was 25.57, 25.71 and 25.86 % in T0, T1 and T_2 groups, respectively, and respective concentration on 90-day of experimental period was 27.71, 28.43 and 27.57 %. But there was no significant difference reported in the concentration of neutrophils of different groups during the entire fortnights. The mean concentration of neutrophils was 26.90, 26.94, and 27.00 % in T0, T1 and T2 groups respectively, but there was no statistical ($P>0.05$) difference reported.

Table 4.10. Effect of nickel supplementation on Neutrophil (%) of Muzaffarnagari sheep

Fortnight	Treatment			SEM	P-value		
	T0	T1	T2		Contrast	Linear	Quadratic
0	25.57	25.71	25.86	1.34	0.913	0.673	1.000
1	26.14	26.29	26.43	1.92	0.952	0.757	1.000
2	27.00	27.14	27.29	1.80	0.946	0.743	1.000
3	26.57	26.86	26.86	1.79	0.928	0.740	0.848
4	27.29	27.29	27.43	1.69	0.980	0.864	0.921
5	28.00	26.86	28.14	1.36	0.133	0.832	0.049
6	27.71	28.43	27.57	1.38	0.439	0.841	0.211
Mean	**26.90**	**26.94**	**27.08**	**1.61**	**0.833**	**0.566**	**0.854**

T0, no nickel group, T1, nickel supplemented group (1.5mg/kg DM); T2, nickel supplemented group (3 mg/kg DM); SEM, Standard error mean

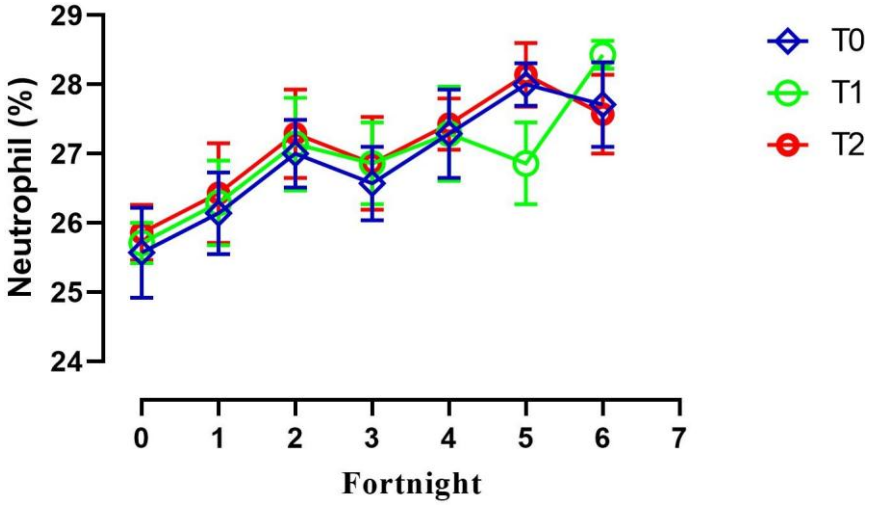


Figure 4.10 Fortnightly changes of neutrophil in Muzaffarnagari sheep supplemented with nickel.

4.3.3. Lymphocyte

The impact of nickel supplementation on the lymphocyte concentration of experimental animals is presented in Table 4.11. And Figure 4.11Nickel supplementation

did not influence the lymphocytes concentration and lymphocyte % was reported statistically similar in the groups on 0, 1, 2, 3, 4 and 5 fortnights. But on the sixth fortnight of study, lymphocyte % was statistically greater in T1 as compared to the T0 group. The overall mean of lymphocytes was reported significantly (P<0.05) higher in T2 than the control group, however, no statistical (P>0.05) difference was observed between T2 vs. T1 and T1 vs. T0 groups.

Table 4.11. Effect of nickel supplementation on Lymphocyte (%) of Muzaffarnagari sheep

Fortnight	Treatment			SEM	P-value		
	T0	T1	T2		Contrast	Linear	Quadratic
0	61.00	61.29	61.43	1.11	0.709	0.422	0.876
1	61.71	61.86	62.00	1.46	0.919	0.685	1.000
2	62.14	62.29	61.86	1.21	0.756	0.628	0.576
3	61.57	63.14	62.43	1.56	0.132	0.260	0.090
4	62.14	62.86	63.57	1.50	0.158	0.058	1.00
5	63.00	62.57	63.71	1.29	0191	0.254	0.152
6	61.71^{a}	63.71^{b}	62.86^{ab}	1.51	0.043	0.136	0.037
Mean	**61.93^{a}**	**62.53^{ab}**	**62.55^{b}**	**1.37**	**0.029**	**0.019**	**0.201**

T0, no nickel group, T1, nickel supplemented group (1.5mg/kg DM); T2, nickel supplemented group (3 mg/kg DM); SEM, Standard error mean

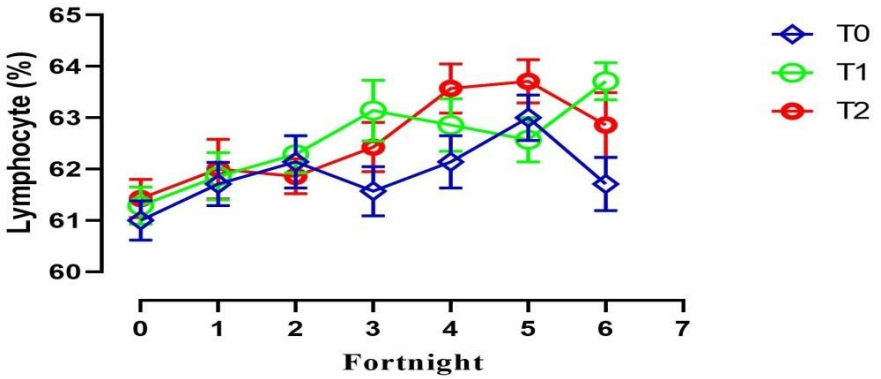


Figure 4.11 Fortnightly changes of lymphocyte in Muzaffarnagari sheep supplemented with nickel

4.3.4. Hemoglobin

The fortnightly data of hemoglobin concentration is given in Table 4.12 and Figure 4.12. The hemoglobin level was 11.86, 11.88 and 11.90 g/dl in T0, T_1 and T_2 groups respectively, on day 0 fortnight of the study period. Whereas, respective concentration was 12.90, 12.51 and 12.65g/dl on the 6^{th} fortnight of trial. However, there was no statistical (P>0.05) difference observed in the overall mean and fortnightly hemoglobin concentration of all three groups.

Table 4.12. Effect of nickel supplementation on Hemoglobin (g/dL) of Muzaffarnagari sheep

Fortnight	Treatment			SEM	P-value		
	T0	T1	T2		Contrast	Linear	Quadratic
0	11.86	11.88	11.90	0.18	0.984	0.861	0.995
1	11.94	12.16	12.18	0.19	0.663	0.419	0.696
2	12.79	11.98	11.99	0.33	0.147	0.091	0.313
3	13.43	12.64	12.83	0.32	0.169	0.166	0.190
4	12.98	13.51	12.99	0.40	0.511	0.980	0.253
5	13.39	13.65	13.70	0.26	0.817	0.560	0.818
6	12.90	12.51	12.65	0.32	0.749	0.627	0.565
Mean	**12.60**	**12.62**	**12.61**	**0.25**	**0.703**	**0.450**	**0.718**

T0, no nickel group, T1, nickel supplemented group (1.5 mg/kg DM); T2, nickel supplemented group (3 mg/kg DM); SEM, Standard error mean

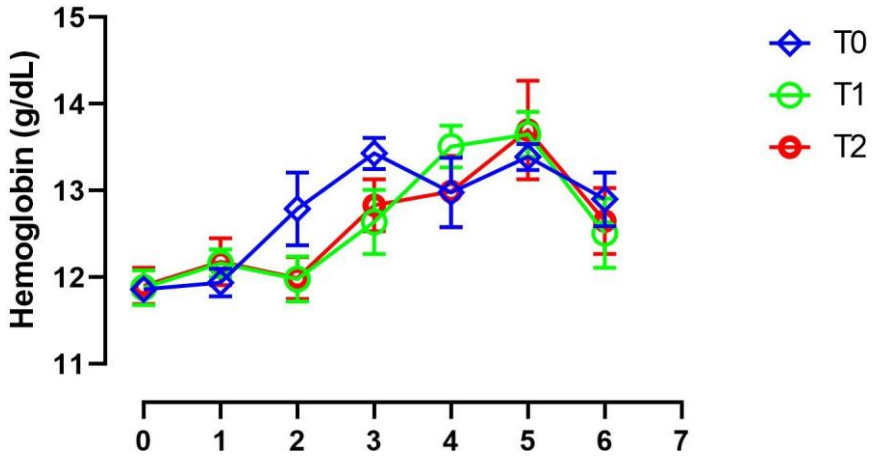


Figure 4.12 Fortnightly changes of hemoglobin in Muzaffarnagari sheep supplemented with nickel

4.3.5. Total immunoglobulin

The impact of nickel supplementation on total immunoglobulin (TIG) of the different groups of Muzaffarnagri sheep is depicted in Table 4.13 and Figure 4.13 The TIG concentration was not varied significantly among groups on 0, 1, 2and 3 fortnights. However, the TIG level was statistically ($P<0.05$) higher in the group receiving 3.0 mg Ni/kg DM than in the group not receiving nickel. Likewise, the overall mean of TIG was statistically ($P<0.005$) greater in T1 (34.00 mg/ml) and T2 (33.81 mg/ml) than in T0 (32.58 mg/ml).

Table 4.13. Effect of nickel supplementation on Total Immunoglobulin (mg/ml) of Muzaffarnagari sheep

Fortnight	Treatment			SEM	P-value		
	T0	T1	T2		Contrast	Linear	Quadratic
0	31.11	31.14	31.16	0.72	0.989	0.884	0.986
1	31.87	31.95	31.99	1.50	0.985	0.868	0.973
2	32.33	32.97	33.02	1.90	0.702	0.456	0.710
3	33.05	34.98	33.73	1.54	0.225	0.186	0.260
4	32.58^{a}	33.97^{ab}	35.08^{b}	1.29	0.010	0.003	0.823
5	33.05^{a}	35.99^{b}	34.00^{ab}	1.83	0.011	0.297	0.004
6	34.04^{a}	36.98^{b}	36.20^{b}	1.06	<0.001	0.001	0.001
Mean	**32.58^{a}**	**34.00^{b}**	**33.81^{b}**	**1.48**	**0.005**	**0.013**	**0.035**

T0, no nickel group, T1, nickel supplemented group (1.5mg/kg DM); T2, nickel supplemented group (3 mg/kg DM); SEM, Standard error mean

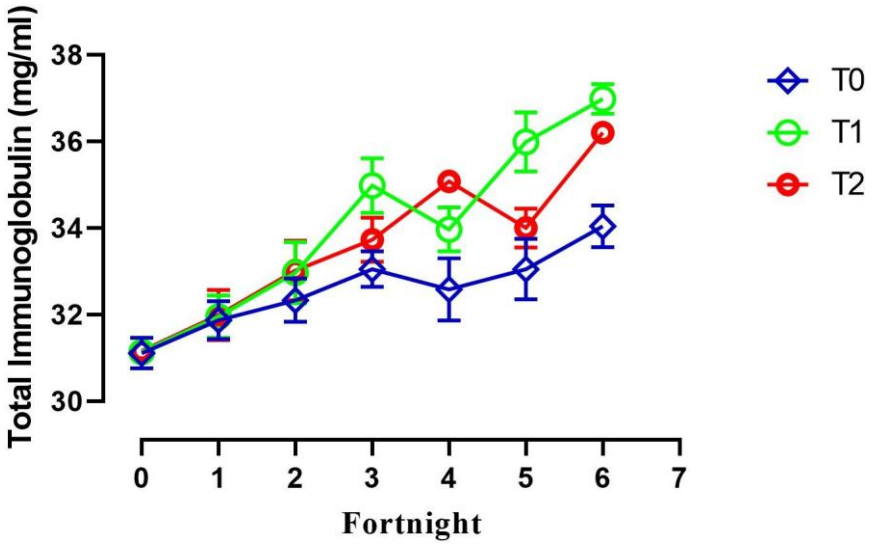


Figure 4.13 Fortnightly changes of total immunoglobulin in Muzaffarnagari sheep supplemented with nickel

4.4. Blood metabolites

4.4.1 Glucose

The data of glucose concentration of all three groups is presented in Table 4.14 and Figure 4.14. Before starting the feeding of nickel, glucose level was 2.57, 2.59 and 2.62mmol/L in T0, T1, and T2, respectively, and the respective values were 2.75, 2.72 and 2.69mmol/L on 6^{th} fortnight of the experimental period. There was no significant (P>0.05) change observed in the concentration of T0, T1 and T2 groups during the entire fortnights of the study period. Similarly, the overall mean of glucose was 2.71, 2.62, and 2.72 in the respective groups and also not varied statistically (P>0.05) among groups.

Table 4.14. Effect of nickel supplementation on glucose (mmol/L) of Muzaffarnagari sheep

Fortnight	Treatment			SEM	P-value		
	T0	T1	T2		Contrast	Linear	Quadratic
0	2.57	2.59	2.62	0.08	0.373	0.171	0.831
1	2.64	2.69	2.73	0.08	0.352	0.688	0.170
2	2.73	2.76	2.78	0.13	0.120	0.126	0.153
3	2.76	2.67	2.79	0.18	0.250	0.106	0.757
4	2.77	2.76	2.78	0.13	0.041	0.024	0.217
5	2.76	2.66	2.67	0.22	0.309	0.141	0.708
6	2.75	2.72	2.69	0.24	0.365	0.178	0.690
Mean	**2.71**	**2.62**	**2.72**	**0.15**	**0.263**	**0.640**	**0.274**

T0, no nickel group, T1, nickel supplemented group (1.5mg/kg DM); T2, nickel supplemented group (3 mg/kg DM); SEM, Standard error mean

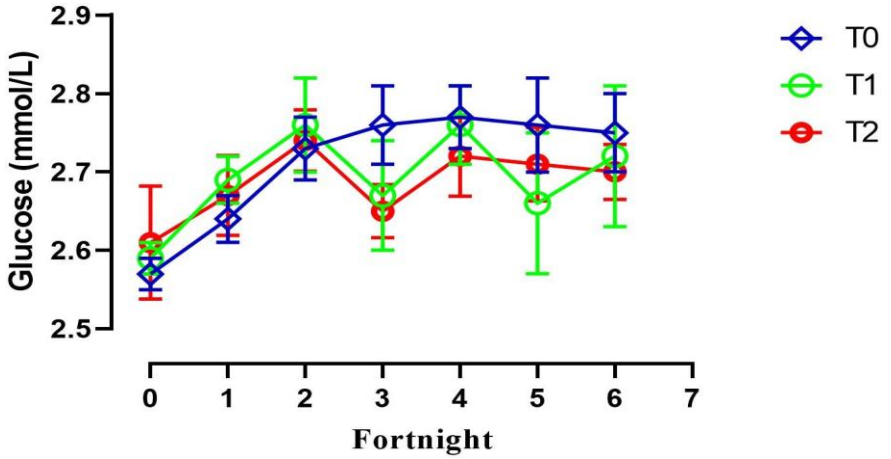


Figure 4.14 Fortnightly changes of glucose in Muzaffarnagari sheep supplemented with nickel

4.4.2 None-esterifies fatty acids

The influence of nickel supplementation on non- esterifies fatty acid (NEFA) of all groups has been displayed in Table 4.15 and Figure 4.15. The NEFA concentration was not affected with supplementation of nickel with dose either 1.5 or 3.0 mg/kg DM and was observed statistically similar in all three groups on entire fortnights of the study period.

Table 4.15. Effect of nickel supplementation on NEFA (µmol/L) of Muzaffarnagari sheep

Fortnight	Treatment			SEM	P-value		
	TO	T1	T2		Contrast	Linear	Quadratic
0	0.16	0.17	0.18	0.05	0.777	0.501	0.840
1	0.18	0.18	0.17	0.04	0.769	0.535	0.719
2	0.15	0.16	0.17	0.04	0.377	0.173	0.835
3	0.16	0.15	0.16	0.03	0.838	0.978	0.558
4	0.17	0.17	0.17	0.04	0.991	0.977	0.895
5	0.16	0.16	0.18	0.03	0.305	0.148	0.620
6	0.18	0.14	0.17	0.06	0.512	0.782	0.267
Mean	**0.16**	**0.16**	**0.17**	**0.04**	**0.542**	**0.396**	**0.478**

T0, no nickel group, T1, nickel supplemented group (1.5 mg/kg DM); T2, nickel supplemented group (3 mg/kg DM); SEM, Standard error mean

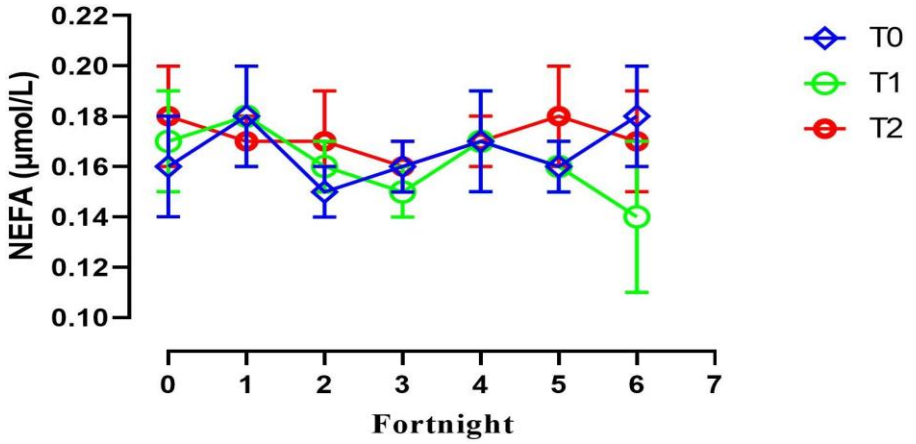


Figure 4.15 Fortnightly changes of non- esterifies fatty acid in Muzaffarnagari sheep supplemented with nickel

4.4.3. Cholesterol

The data of cholesterol of all three groups is presented in table 4.16 and Figure 4.16. The mean value of cholesterol was 3.04, 3.01 and 3.03 mmol/L in T0, T1 and T2 groups, respectively and did not vary significantly among groups. Likewise, statistically (P>0.05) similar concentration was observed in all three groups of sheep on 0, 1, 2, 3, 4, 5 and 6 fortnight of the study period.

Table 4.16. Effect of nickel supplementation on cholesterol (mmol/L) of Muzaffarnagari sheep

Fortnight	Treatment			SEM	P-value		
	T0	T1	T2		Contrast	Linear	Quadratic
0	2.85	2.81	2.87	0.23	0.876	0.850	0.638
1	3.01	2.99	3.04	0.27	0.937	0.879	0.749
2	3.06	3.00	3.07	0.28	0.871	0.939	0.608
3	2.99	2.90	3.14	0.37	0.427	0.411	0.313
4	3.14	3.13	3.16	0.35	0.991	0.937	0.913
5	3.16	3.10	3.18	0.38	0.913	0.916	0.683
6	3.15	3.12	3.15	0.21	0.963	0.978	0.788
Mean	**3.04**	**3.01**	**3.03**	**0.29**	**0.405**	**0.547**	**0.230**

T0, no nickel group, T1, nickel supplemented group (1.5mg/kg DM); T2, nickel supplemented group (3.0mg/kg DM); SEM, Standard error mean

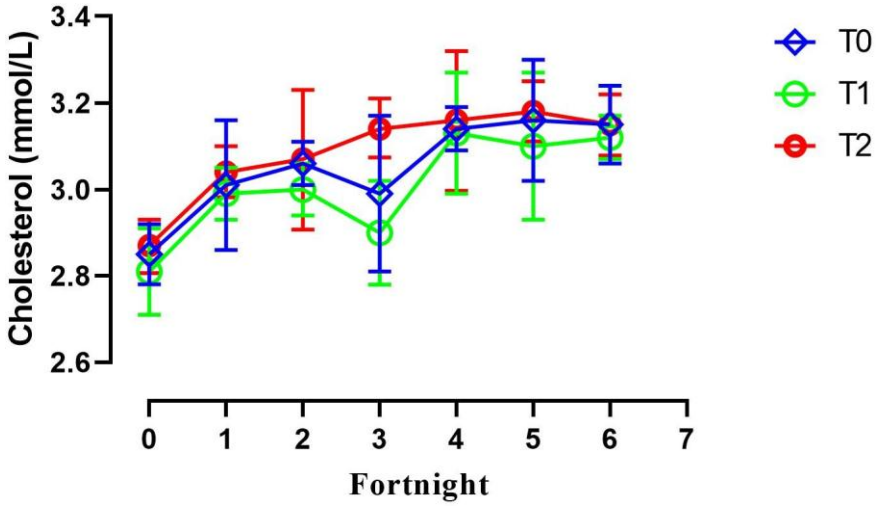


Figure 4.16 Fortnightly changes of cholesterol in Muzaffarnagari sheep supplemented with nickel

4.4.4. HDL- cholesterol

The plasma on concentration of HDL cholesterol of all three groups is reported in Table 4.17. And Figure 4.17. The nickel supplementation did not influence the HDL cholesterol concentration and was observed significantly (P>0.05) similar in all three groups on all the fortnights of the study period. Moreover, the overall mean of HDL cholesterol was reported 1.58 (T0), 1.60, (T1) and 1.61, (T2), which indicated that the concentration of HDL cholesterol was statistically (P>0.05) similar.

Table 4.17. Effect of nickel supplementation on HDL cholesterol (mmol/L) of Muzaffarnagari sheep

Fortnight	Treatment			SEM	P-value		
	T0	T1	T2		Contrast	Linear	Quadratic
0	1.53	1.55	1.58	0.11	0.648	0.358	0.987
1	1.56	1.58	1.61	0.10	0.462	0.227	0.831
2	1.59	1.60	1.57	0.13	0.879	0.838	0.646
3	1.58	1.64	1.63	0.13	0.495	0.367	0.445
4	1.62	1.55	1.62	0.17	0.653	0.959	0.363
5	1.61	1.65	1.64	0.11	0.764	0.594	0.622
6	1.60	1.64	1.59	0.12	0.565	0.861	0.298
Mean	**1.58**	**1.60**	**1.61**	**0.12**	**0.514**	**0.296**	**0.627**

T0, no nickel group, T1, nickel supplemented group (1.5mg/kg DM); T2, nickel supplemented group (3 mg/kg DM); SEM, Standard error mean

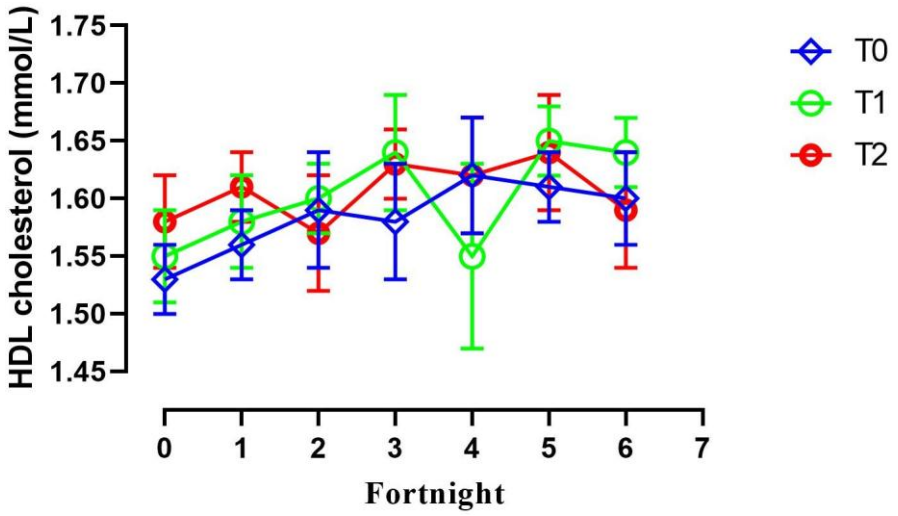


Figure 4.17 Fortnightly changes of HDL cholesterol in Muzaffarnagari sheep supplemented with nickel

The discussion on the current study entitled **"Effect of nickel supplementation on growth performance, antioxidant and blood metabolites of Muzaffarnagri sheep"** has been presented in this chapter.

Growth performance

Dietary fed of nickel with doses of 1.5 or 3.0 mg/kg DM Muzaffarnagri sheep did not affect the body weight. Similar findings were reported by Pandey *et al.* (1999) who reported no change in body weight of mice with the supplementation of nickel. However, Smialowicz *et al.,* (1987) in rats observed a decrease in body weights, but Milne *et al.* (1990) reported that increase the body weight in lambs with nickel supplementation. The present study denoted that body weight gain was improved with nickel supplementation. In agreement with the present findings, Bersenyi *et al.* (2004) in broiler chicken and Dunnick *et al.* (1989) on the rats were reported higher body weight gain with nickel supplementation. Dietary nickel did not affect feed consumption was consistent with the findings of previous studies reported by Kirchgessner *et al*. (1977) in pig, Samal and Mishra, (2011) in the rats and Adams *et al.* (1992) in sheep. Feed conversion ratio was declined with supplementation of nickel as reported in the present study. Elevated FCR in nickel supplemented groups is agreeing with the previous study reported by Spears, (1984) in rats and Singh *et al.* (2019) in heifers.

5.2 Antioxidant status

Dietary fed nickel did not affect the concentration of superoxide dismutase. However, Das *et al.,* (2001), Farid *et al.,* (2012), Misra *et al*. (1991) and Terpilowska and Siwicki (2019) reported that the SOD concentration was decreased in nickel treated groups. Catalase was increased in sheep fed on diets with nickel supplementation @ 1.5 and 3.0 mg/kg DM. The results are in agreement with Terpilowska and Siwicki, (2019) in mice. Thamizhan

(2020) also found that catalase activity was higher in Murrah buffalo calves. Kong *et al.* (2019) reported higher catalase activity in nickel treated adult male rats. Total antioxidant activity was not affected with nickel supplementation. The result of the present study is in accordance with the Liapi *et al.,* (2011) in male Wistar rats. Whereas, Al-Humadi and H. W, (2015) reported a lower circulating concentration of TAA in the blood of rat supplemented with nickel. Thiobarbutric acid reactive substance was lowered in nickel treated sheep as reported in the present study. Similar results were observed by other researchers, Prasad *et al.* (2006) and Chen *et al*. (1999) both reported that constant result was found in rats of the present study.

5.3 Immune status

Dietary fed of nickel did not affect total leukocyte count according to the results of this research. A similar report has come from Hujanen *et al.* (1995) in the rat. The circulating concentration of neutrophils was unaffected in the treatments supplemented with nickel. The results of the present study are in accordance with the Osman *et al.* (2012) in the rat. Nickel supplementation has a positive effect in the improvement of the circulating concentration of lymphocytes in the blood. In line to the present study Zalkind *et al*. (1998), Yadav *et al.* (2019 and Cederbrant *et al*. (2003) reported higher lymphocytes in nickel treated groups. The hemoglobin concentration was not affected with dietary nickel supplementation. Similar observation was also reported by Nilsen *et al.* (1980) in rats. The circulating concentration of total immunoglobulin was increased with the dietary supplementation nickel. In agreement with present study, Hashem *et al*. (2019) reported that total immunoglobulin was increased in rat fed nickel supplementation.

5.4 Blood metabolites

In present study, the circulating concentration of plasma glucose was affected with the supplementation of nickel in growing sheep. The glucose concentration in the present study was similar to the range reported by Horak *et al*. (1978). In contrast with the present findings; Peligero *et al*. (1985) reported nickel supplementation improved the glucose concentration in rats. Supplementation of nickel in sheep did not affect the plasma levels of NEFA in the present study. It is in confirmation with the results of earlier findings reported by Singh *et al.* (2016). No impact of nickel supplementation on the cholesterol concentration of sheep was reported in the current study. Dissimilar results were observed by another researcher, Das *et al.* (2006), Gupta *et al* (2008) in rat. Plasma concentration of HDL cholesterol was unaltered with nickel supplementation. The results of the present study are not in accordance with the Das *et al*. (2001) in rat Singh *et al*. (2019) in heifer and Das *et al.* (2006) in rat.

The present investigation, **''Effect of nickel supplementation on growth performance, antioxidant and blood metabolites of Muzaffarnagri sheep"** was carried out.

6.1 Summary

For the present study, Twenty-one Muzaffarnagri sheep were selected from the herd maintained at Livestock Research Complex-2, SVPUA&T, Meerut, UP, India, for the period of 90 days. Experimental animals were divided into three groups (n=7) on body weight (30.45±3.67 kg) and age (9.86±0.59 months) basis. 1. Group 1 (T0) was acted as a control and fed on the basal diet without any nickel supplementation. Experimental animals either received a basal diet devoid of supplemental Ni (T0) or were supplemented with 1.5 mg (T1) and 3.0 mg (T2) of Ni/kg DM for the period of 90 days. The nutrient requirements of experimental sheep were met by feeding total mixed ration (TMR) consisted of concentrate: green berseem fodder: wheat straw in the proportion of 45:35:20 following NRC (2001) guidelines. Blood was sampled at fortnightly intervals from the jugular vein in the EDTA coated Vacationer tube at 07.00 a.m. before feeding and watering. Fraction of blood was used in the estimation of hemoglobin, total leukocyte counts, lymphocyte and neutrophil. The remaining blood sample was then centrifuged at 3000 rpm for 30 min for separating plasma. The plasma was kept in Eppendorf tubes and stored at -20 ^{o}C till further analysis of total immunoglobulin, total antioxidant activity, thiobarbituric acid reactive substance and glucose, non-esterifies fatty acids, cholesterol, HDL-cholesterol. The hematocrit was used in the preparation of hemolysate, which is utilized in the analysis of superoxide dismutase and catalase. Body weight and feed consumption was recorded on the day of blood collection with the help of digital

electronic balance. Body weight gain and feed conversion ratio were calculated from the body weight and feed consumption.

Nickel supplementation did not affect the body weight and was reported statistically similar in all three groups on each fortnight of the study period (Table4.1; Figure 4.1).The BWG was statistically greater in the group receiving 3.0 mg nickel/kg DM than 1.5 mg nickel/kg DM supplemented and non-supplemented groups. Feed consumption was not affected with the supplementation of nickel and was reported similar in all three groups (Table 4.3). The feed conversion ratio was significantly lower in T2 groups as compared to T0 and T1 groups of sheep. The SOD activity was not affected with supplemental nickel and remained similar statistically ($P>0.05$) in all the groups on all the fortnights of the study period. Likewise, the mean activity of SOD was also not varied significantly ($P>0.05$) among the groups (Table 4.5). Mean catalase activity was shown a statistical difference and was observed significantly ($P<0.05$) lower in T2 as compared to control and T1 groups (Table 4.6).Total antioxidant activity was not affected by nickel supplementation and was reported statistically ($P>0.05$) similar in all three groups on entire fortnights of the study. The mean value of TBARS was 1.38, 1.35 and 1.33 nmol/ml in the control, T_1, and T_2 groups, respectively, and the observed values were statistically similar in the groups (Table 4.8). There was no significant difference was observed in the overall mean of TLC of all three groups and the value was 7.96, 7.98, and 7.97($\times 10^3/\mu l$) in T0, T1 and T2 groups, respectively (Table 4.9). The mean concentration of neutrophils was 26.90, 26.94, and 27.00 % in T0, T1 and T2 groups respectively, but there was no statistical ($P>0.05$) difference reported (Table 4.10). The lymphocytes % was reported statistically similar in the groups on 0, 1, 2, 3, 4 and 5 fortnights. The mean value of lymphocytes was significantly ($P<0.05$) higher in T2 than

in the control group (Table 4.11).There was no statistical (P>0.05) difference observed in the overall mean and fortnightly hemoglobin concentration of all three groups (Table 4.12). TIG level was statistically (P<0.05) higher in the nickel-treated groups than in the control (Table 4.13). There was no significant (P>0.05) change observed in the concentration glucose of T0, T1 and T2 groups during the entire fortnights of the study period (Table 4.14). The NEFA concentration was not affected with supplementation of nickel with dose 1.5 or 3.0 mg/kg DM and was observed statistically similar in all three groups on entire fortnights of the study period (Table 4.15; Figure 4.15). The mean value of cholesterol was 3.04, 3.01 and 3.03 mmol/L in T0, T1 and T2 groups, respectively and did not vary significantly among groups (Table 4.16). The nickel supplementation did not affect the HDL cholesterol concentration and was observed significantly (P>0.05) similar in all three groups on all the fortnights of the study period (Table 4.17).

Conclusions:-

It may be concluded that supplementation of nickel beneficial for sheep Body weight gain, lymphocytes, total immunoglobulin and catalase were increased, and feed conversion ratio was decreased with the supplementation of nickel. This indicates that nickel supplementation improved the growth performance and immunity of sheep.

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Abstract

The aim of this study to investigate the impact of nickel (Ni) supplementation on growth performance, antioxidant and blood metabolites. Twenty-one Muzaffarnagri sheep was selected from the herd maintained at Livestock Research Complex-2, Experimental animals were blocked into three groups (n=7) on body weight (30.45±3.67 kg) and age (9.86±0.59 months) basis. Experimental animals either received a basal diet devoid of supplemental Ni (T0) or were supplemented with 1.5 mg (T1) and 3.0 mg (T2) of Ni/kg DM for the period of 90 days. Body weight and feed consumption were recorded at fortnightly intervals, and body weight gain and feed conversion ratio were calculated form body weight and feed consumption. In whole blood, total leukocyte counts (TLC), lymphocyte and neutrophil counts, and hemoglobin were measured. Plasma was tested for total antioxidant activity (TAA), thiobarbituric acid reactive substance (TBARS), total immunoglobulin (TIG), glucose, non-esterifies fatty acid (NEFA), total cholesterol, and HDL-cholesterol. Catalase and superoxide dismutase (SOD) was analyzed in hematocrit. Nickel supplementation had no effect on body weight and feed consumption in all three groups was statistically similar at the end of each week of the study. The BWG in the T2 group was statistically ($P<0.05$) greater than in the other groups. The feed conversion ratio was significantly lower in T2 groups as compared to T0 and T1 groups of sheep. Total antioxidant activity, SOD activity and TBARS were statistically similar ($P>0.05$) in all groups on all fortnights of the study period. Catalase was observed significantly ($P<0.05$) lower in T2 as compared to control and T1 groups. There was no significant difference observed in hemoglobin, TLC and neutrophil concentration of T0, T1 and T2 groups. The lymphocytes was significantly ($P<0.05$) higher in T2 than in the control group. TIG level was statistically ($P<0.05$) higher in the nickel-treated groups (1.5 mg Ni/kg DM) than in the control. The plasma concentration of NEFA, total cholesterol, HDL cholesterol was not affected with the supplementation of nickel with any dose and was reported similar values in all three groups. The study concluded that nickel supplementation was improved growth performance and immunity of the sheep.

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