ADDIS ABABA UNIVERSITY

Center for Food Science and Nutrition

College of Natural Science

Effect of altitude – induced hypoxia and iron supplementation on fetal growth restriction and cognitive development of Wistar rats

By: Yirgalem Tadesse Desta

Advisor: Dr. Kaleab Baye (Ph.D.)

A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Food Science and Nutrition

Jun, 2020

Addis Ababa, Ethiopia

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SCHOOL OF GRADUATE STUDIES
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This is to certify that the thesis prepared by **Yirgalem Tadesse Desta** entitled '***Effect of altitude – induced hypoxia and iron supplementation on fetal growth restriction and cognitive development of Wistar rats'*** and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Food Science and Nutrition of Addis Ababa University and meets the accepted standards with respect to originality and quality.

Signed by the examining committee

Approved by	**Signature**	**Date**
1. **Dr. Abebe Haile (Ph.D.) (External Examiner)**	______	**Sep 15, 2020**
2. **Dr. Aynadis Tamene(Ph.D.) (Internal Examiner)**	______	______
3. **Dr. Paulos Getachew(Ph.D.) (Chairman)**	______	______

Advisors

4. **Dr. Kaleab Baye (Ph.D.)** ______ **Sep 05, 2020**

Declaration

I hereby declare that the thesis entitled *"**Effect of altitude – induced hypoxia and iron supplementation on fetal growth restriction and cognitive development of Wistar rats**"* has been carried out by me under the supervision of Dr. Kaleab Baye (Ph.D.), Addis Ababa University Center for Food Science and Nutrition Studies as a part of Masters of Science Program. I further declare that this thesis is my original work and has not been presented for a degree of master in any other University and that all the sources and materials used for the thesis have been properly acknowledged.

Declared by:- **Yirgalem Tadesse Desta** Signature ________________ Date Aug, 2020

This is to certify that the above declaration made by the candidate is correct to the best of my knowledge as an advisor.

Approved by Advisor:- **Dr. Kaleab Baye (Ph.D.)** Signature________________Date Aug,

Acknowledgments!

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LIST OF ACRONYMS AND ABBREVIATIONS

AAU:	Addis Ababa University
AIN:	American Institute of Nutrition
ANOVA:	Analysis Variance
CRP:	C- Reactive protein
CSA:	Central Statistical Agency
EDHS:	Ethiopia Demographic and Health Survey
EPHI:	Ethiopian Public Health Institute
HA:	High Altitude
HAZ:	height- for-age Z-score
HB:	Hemoglobin
IDA:	Iron deficiency anemia
IFA:	Iron Folic Acid Supplement
IUGR:	Intrauterine growth restriction
LA:	Low Altitude
MWM:	Morris Water Maize
PEM:	protein energy malnutrition
SD:	Standard Deviation
SNNP:	Southern Nation & Nationality Peoples
SPSS:	Statistical Software Package System
WHO:	World Health Organization

Abstract

Stunting is associated with increased morbidity and mortality, reduced neurocognitive function, decreased learning capacity and productivity as well as poor long-term health outcomes. A large share of the drivers of stunting remains unidentified. Although hypoxia-related to pregnancy at high altitude can potentially lead to intrauterine growth restriction, little is known about its potential contribution to the stunting burden. The objective of this study is to investigate the effect of altitude-induced hypoxia on fetal growth restriction and cognitive development of wistar rats. The study was conducted in two different altitude sites of Ethiopia one at Debre-Berhan University (DBU) 2,840 mas and at Allage Agricultural college 1543 mas. At initial we use (n=32) and their two generations of rats were used for the experiments, which were randomly assigned into treatment (AIN-93G diet containing 75 mg/kg) iron and control group (standard AIN-93 G: 35 mg Fe/kg). Food and water intake (daily for 1st generation) weight and length from birth (once a week) and hemoglobin concentrations were measured. Cognition was tested using the Morris Water Maize (MWM) and the electric foot shock tests, which were conducted when the rats turned 45 days old. Feed intake was higher in higher altitude, while water intake was higher in the lower altitude (P< 0.05). The birth weight of the pups ranged between 4.5g to 7.2g. Birth length was significantly lower at higher altitude than in lower altitude (P< 0.05). Irrespective of altitude, birth weight and birth length was higher in the treatment than in the control group. In all cases, the hemoglobin concentration of the treatment group was significantly higher than the control group (P< 0.05). The time taken to reach the goal in the water maze experiment was similar (~37 seconds) for both groups, However, among the treatment groups, where rats in lower altitude took less time to reach the target than their high altitude counterparts rats in the control group. Our findings highlight that birth at high altitude increases the risk of low birth weight/length, with serious consequences on cognitive performance. However, treatment with higher dosage of iron during gestation can counter the adverse effects of hypoxia-related adverse effect in pregnancies and growth at higher altitude.

Keywords: *stunting, Hypoxia, leaner growth, birth weight, birth length, Cognation*

1. CHAPTER ONE: INTRODUCTION

1.1 Background

Stunting or poor linear growth is defined as a child with a height-for-age Z-score (HAZ) <-2 SD below the median of a reference height-for-age standard (De Onis et al., 2013). Stunting is associated with increased morbidity and mortality, reduced neurocognitive function, decreased learning capacity and productivity; and poor long-term health outcomes, putting stunted children at risk of chronic disease in adulthood (Black et al. 2013). Childhood stunting varies not only across various regions of the world, but also within and between local authorities, regional space dimensions and/or countries (Kinyoki et al., 2020). In 2012, the World Health Organization adopted a resolution on maternal, infant and young child nutrition that included a global target to reduce the number of stunted under-five children by 40% by 2025. The target was based on analyses of time series data from 148 countries and national success stories in tackling under nutrition (Walker et al., 2016). The global target translates to a 3.9% reduction per year and implies decreasing the number of stunted children from 171 million in 2010 to about 100 million in 2025.

However, at current rates of progress, there will be 127 million stunted children by 2025, that is, 27 million more than the target or a reduction of only 26% (De Onis et al., 2013). Despite reductions in the prevalence of stunting globally, stunting remains a serious public health concern in Sub-Saharan Africa where the number of stunted children is rising (Wirth et al., 2017). Although Ethiopia has made steady progress in reducing stunting from 58% in 2000 to 38% in 2016, a much faster reduction rate will be needed to achieve the WHO resolutions. The prevalence of underweight has consistently decreased from 41% to 24% over the 16-year period (CSA, 2016), On the other hand, the prevalence of wasting changed little over the same period, with a wasting rate of 10% in 2016, which about the same rate of wasting in 2011.

High altitude reduces birth weights, averaging a 100-g fall per 1000 m elevation gain, as the result of restriction of third trimester fetal growth. Intrauterine growth restriction (IUGR) raises neonatal or infant mortality at low as well as at high altitude, but existing studies are unclear as to whether IUGR-

Specific mortality at high altitude is similar to, less than, or greater than at low altitude. Pregnancy increases maternal ventilation and raises arterial O2 saturation at high altitude, which helps to protect against altitude-associated IUGR (Moore, Charles, & Julian, 2011). Chronic hypoxia interferes with the maternal circulatory adjustments to pregnancy such that blood volume is lower and the rise in cardiac output diminished compared with sea level. The growth and remodeling of the uterine artery and other uteroplacental vessels is incomplete at high compared with low altitude, with the result that there is less redistribution of common iliac flow from the external iliac to the uterine arteries and lower uterine artery blood flow near term(Sandovici, Hoelle, Consta, & Angiolini, 2012) . Adaptations in multigenerational high altitude populations (e.g., Andeans and Tibetans) permit higher uterine artery blood flows and protect against altitude-associated IUGR (Julian, 2011), (Manuscript, 2012).

The impact of nutrition and/or iron supplements on further optimization of these hypoxic adaptations at low-moderate altitudes is only an emerging topic (Giussani, 2016). Iron is an essential nutrient throughout the human life cycle, but particularly for the developing fetus, the neonate, and in early childhood. Before birth, iron needs are met through maternal iron transfer, and after birth, through dietary sources Worldwide, iron deficiency remains one of the most common micronutrient deficiencies in children, affecting an estimated 43% of the global population. Although a child with iron deficiency will continue to grow, depletion of tissue iron stores will cause specific clinical symptoms (Cerami, 2017).

Furthermore, the safest way to address the possible increase in iron deficiency anemia associated with altitude exposure is via the consumption of iron-rich foods rather than high-dose iron supplements (Walter et al., 2002). Meanwhile, many other important questions regarding nutrition and altitude training remain to be answered. Sufficient iron stores are required to support a hypoxic-mediated increase in heme synthesis and iron-dependent enzyme production during prolonged altitude exposure. Indeed, altitude exposure is associated with a three- to five-fold increase in erythropoiesis, with erythroid iron uptake approaching 100% of its maximal capacity during the first few days of adaptation (Akhtar, Anjum, & Anjum, 2011).

There for the aim of this study is to have animal based evidence of the effect of Effect of altitude induced hypoxia and iron supplementation on fetal growth restriction and cognitive development on Wistar rats in two separate study site having different altitude measurements.

1.2 Statement of the problem

Much more substantial declines in stunting prevalence need to be witnessed to achieve the WHO targets. To this end, scaling up proven interventions needs to happen much faster. However, scaling up nutrition-specific interventions to universal coverage level is only expected to address 26% of the stunting cases. To address the remaining cases, nutrition-sensitive interventions have been recommended. Within the set of nutrition-specific interventions, very few are targeting linear growth faltering at birth that can contribute up to 20% of growth faltering. What leads to growth faltering in utero, and which interventions are likely to address it remains largely unknown. One such factor that requires attention is pregnancy in high altitude areas where pregnant women will be exposed to altitude-induced hypoxia.

In the case of pregnant women, the maternal side, one of the greatest physiologic challenges is to maintain an adequate supply of oxygenated blood to the utero placental circulation for fetal development. This challenge of oxygen transport is magnified under conditions of limited oxygen availability, such as hypoxia from high-altitude exposure or pathologic states such as pulmonary disease or anemia (Julian, 2011). Would this altitude-induced hypoxia lead to higher rates of growth faltering in high altitude areas? Would iron supplementation need to be adapted to altitude? Would iron supplementation in high altitude areas lead to lower growth faltering in utero? All these questions are difficult to answer in human models, particularly because some level of adaptation has been documented for communities that have ancestors that lived in high altitude and thus require at first animal studies that can be performed over various generations.

1.3 Objectives

1.3.1. General Objective

- To investigate the Effect of altitude - induced hypoxia on fetal growth restriction and cognitive development of wistar rat.

1.3.2. Specific Objectives

- Determine the association between altitude and linear growth faltering of wistar rats
- Investigate the effect of iron supplementation on linear growth at high and low altitude
- Investigate the intergenerational effect of altitude-induced hypoxia on linear growth and cognitive development

2. CHAPTER TWO: LITERATURE REVIEW

2.1 Stunting

Stunting or poor linear growth is defined as a child with a height-for- age Z-score (HAZ) less than minus two standard deviations (<−2 SD) below the median of a reference height-for-age standard (Haile, Azage, Mola, & Rainey, 2016). Stunting reflects linear growth retardation accumulated before and after birth (Walker et al., 2016). Worldwide, stunting affects nearly one-third of children under 5 years of age, with the prevalence being higher in low-resource countries in sub-Saharan Africa and South Asia (Umeta et al., 2003).

Stunting varies childhood is not only across various regions of the world but also within and between local authorities, regional space dimensions and/or countries (Adekanmbi et al.,1013). In 2012, the World Health Organization adopted a resolution on maternal, infant and young child nutrition that included a global target to reduce by 40% the number of stunted under-five children by 2025(Woodruff et al., 2017). The target was based on analyses of time series data from 148 countries and national success stories in tackling under nutrition (Walker et al., 2016).

Reducing the prevalence of stunting is one of the World Health Assembly's Nutrition Targets for 2025 (40% reduction in the number of under five children stunted) (WHO 2012). Globally, stunting has declined by 1.8% annually between 1995 and 2010, with some countries observing annual reductions of 3.9% or higher in this time period (de Onis et al. 2013)

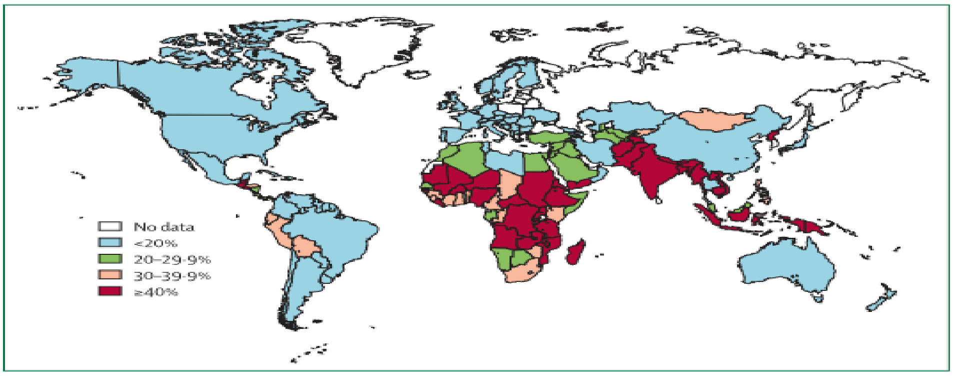

Figure 1: Prevalence of stunting in children less than 5 years

Source ; (Black et al., 2008)

2.2 Consequences of stunting

Adequate provision of nutrients is crucial to ensure good physical & mental development as well as for long-term health. Under nutrition, accounts for 35% of all deaths among under five children. More than 2 million under-five children die each year due to under nutrition (Umeta, West, Verhoef, Haidar, & Hautvast, 2018). Under- nutrition refers to a state resulting from a relative or absolute deficiency of one or more essential nutrients (Fikadu et al., 2014).

2.2.1. Short-term consequences of stunting

Stunting reflects a failure to receive adequate nutrition over a long period of time, and is affected by both recurrent and chronic illness (Haile et al., 2016).

It is estimated that malnutrition, in particular under-nutrition, is the cause of more than half of all infant and child morbidity in sub-Saharan Africa (African & Journal, 2005). Poor nutritional status in developing countries is often associated with fetal growth retardation, which often results from low maternal food intake, strenuous physical work, ignorance, and infection during pregnancy (Walker et al., 2016). It is also associated with increased morbidity and mortality; reduced neuron cognitive function, deprived attention, memory impairment (Fikadu et al., 2014),decreased learning capacity and productivity; and poor long-term health outcomes of later in life, putting them at risk of chronic disease in adulthood (Wirth et al., 2017)

In the short-term, the consequences of stunting include an increased risk of, stillbirth abortion and death due to infectious diseases and poor psychomotor and mental development (Abubakar et al. 2010; Woodruff et al., 2017).

2.2.2. Long-term health consequences of stunting

In the long term, childhood stunting has been linked to obesity and diabetes (Bhargava et al. 2004) and lower human capital in adulthood (Victora et al. 2008). However, children who are stunted usually grow up to be stunted adults (Martorell et al. 1994). An opportunity exists to make up some of the height deficit during adolescence because stunted children often experience a delay in skeletal maturation, lengthening the total period of time for growth in height and become grow to maternal stunting (Dewey & Begum, 2011).

Maternal stunting can restrict uterine blood flow and growth of the uterus, placenta, and fetus. Intrauterine growth restriction (IUGR) is associated with many adverse fetal and neonatal outcomes (Black et al. 2008). During pregnancy, IUGR may lead to chronic fetal distress or fetal death. If born alive, the growth-restricted infant is at higher risk for serious medical complications (Black et al. 2008). Infants with IUGR often suffer from delayed neurological and intellectual development, and their deficit in height generally persists to adulthood.

Maternal stunting is consistently associated with an elevated risk of prenatal mortality (stillbirths and deaths during the first 7 days after birth) (Lawn et al. 2009), mostly related to obstructed labour resulting from a narrower pelvis in short women. In a hospital-based study in Nigeria, obstructed labour accounted for 53% of prenatal mortality (Omole-Ohonsi & Ashimi 2007).

Prenatal mortality from obstructed labour is largely the result of birth asphyxia. Mothers with height shorter than 145 cm are more likely to have an infant with birth asphyxia (Lee et al. 2009). Globally, birth asphyxia accounts for 23% of the four million neonatal deaths each year (Lawn et al. 2005). An estimated one million children who survive birth asphyxia live with chronic neurodevelopment disorders, including cerebral palsy, mental retardation and learning disabilities (World Health Organization 2005)

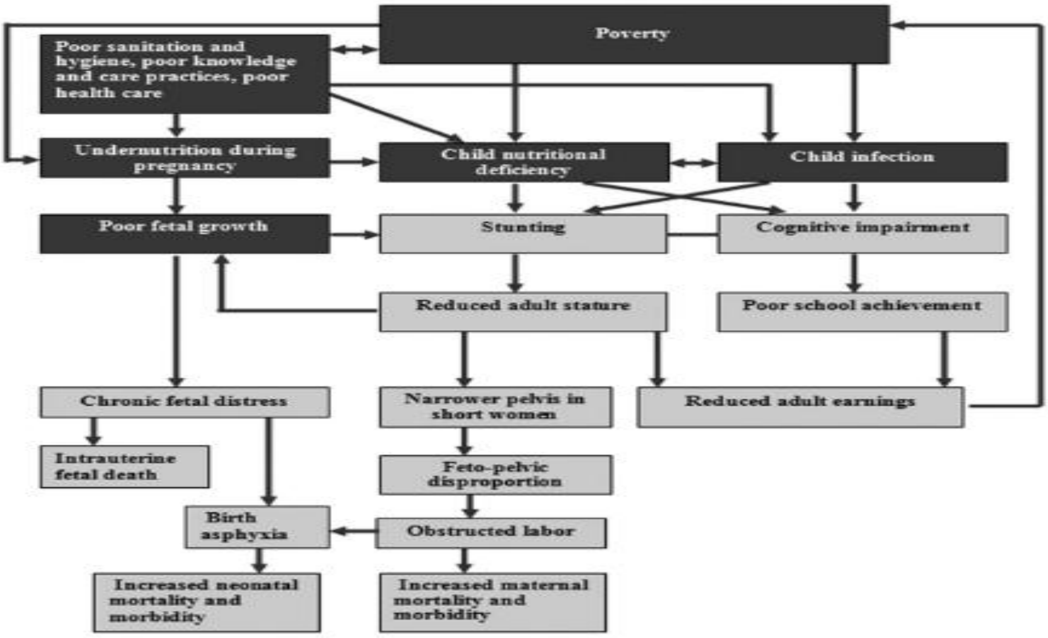

Figure 2: Potential causal pathways for long-term consequences of stunting

source: (Dewey & Begum, 2011)

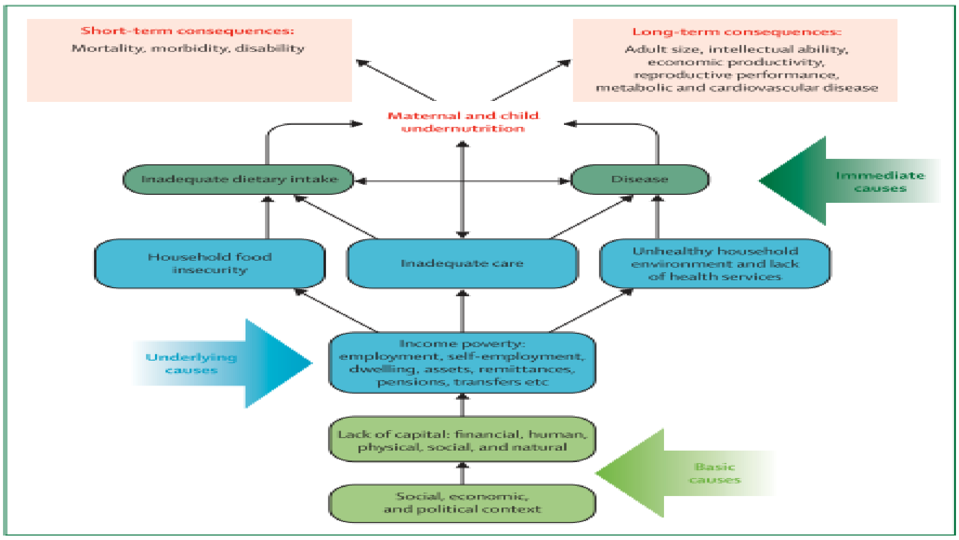

Figure 3: Framework of under nutrition and its short-term and long-term consequences

Source; (Black et al., 2008)

2.3 Risk factors for stunting

Chronic malnutrition among Ethiopian children is very high, with more than one in two children (52%), being stunted and more than one in four children (26%) being severely stunted (CSA, 2000). The toddlers appeared to be the most (60%) susceptible to stunting which concurs with the findings of national and other pocket surveys conducted in the country (CSA, 2000).

The proportion of wasted children was highest in the 13-24 month age group, which is highly likely to be an indication of inadequate complementary feeding and exposure to infections during the weaning period. The high level of malnutrition observed could be attributed to various socio-economic barriers that exist in the community. Harmful traditional practices such as early marriage and inequitable intra-household food distribution, ascribing to food taboos; low maternal nutrition are other barriers that contribute to the high level of malnutrition (African & Journal, 2005).

2.3.1 Community and societal factors

The WHO Stunting Framework labels community and social factors as ‘contextual’ and categorizes them into six groups: (a) economy of household and the community; (b) health system; (c) education and knowledge; (d) society and social culture; (e) agriculture and food systems; and (f) availability of clean water, hygiene of living environment. The current evidence for association between these factors and stunting is seems limited but has a significant contribution for the prevalence of stunting (Stewart et al. 2013), and many context factors (e.g. population density, per-capita national income, level of democracy are calculated at the national level and are thus not applicable for analysis at the household or community level (Pridmore & Hill 2009).

Cultural beliefs, norms and social support networks are posited to contribute to poor feeding and dietary patterns. Women's status in particular is seen as a cultural factor that can influence child health, and it is frequently assessed using data on household decision- making relating to cooking, purchase of food and household items, ability to take short trips to market or relatives' homes, etc. In Ethiopia, women with high decision-making autonomy are less likely to have a low BMI themselves (Tebekaw, 2011).

2.3.2 Breastfeeding

Maternal under nutrition has little effect on the volume or composition of breast milk unless malnutrition is severe. The concentration of some micronutrients (vitamin A, iodine, thiamin, riboflavin, pyridoxine, and cobalamin) in breast milk is dependent on maternal status and intake, so the risk of infant depletion is increased by maternal deficiency (Bryce, Morris, & Victora, 2008).This factor is most evident in the case of vitamin A, where the content in breast milk is the main determinant of infant status because stores are low at birth. Maternal supplementation with these micronutrients increases the amount secreted in breast milk, which can improve infant status(Black et al., 2008).

In Ethiopia, 52 and 80% of the newborns are put to breast within the first hour and 1 day after birth, respectively (CSA, 2012), according to Teshome et al.2009 observed that, increased prevalence of stunting when breastfeeding was continued past 12months. One study from northern Ethiopia showed that children not given colostrum were twice as likely to be stunted as children who received it (Teshome et al. 2009). In the SNNP region, Fikadu et al. (2014) found that children 24–59months of age who were exclusively breastfed for the first 6months of life were less likely to be stunted than non-exclusively breastfeed children(Wirth et al., 2017).

2.3.3 Inadequate complementary feeding

Inadequate nutrient intake during early childhood is recognized as a causal factor of growth failure, but evidence on specific nutrients other than zinc and protein is limited and mixed (Branca & Ferrari 2002). Related to poor quality foods, inadequate feeding practices and food and water safety (Branca & Ferrari 2002), studies shows that a wide range of compounds, such as phytate, poly- phenols, inhibitors of trypsin and chymotrypsin and lectins (Roos et al. 2013), haemaglutinins, goitrogens, saponins and oxalates they are highly associated with stunting (Melese 2013). Unfortunately, no studies could be found examining the effect of consumption of these compounds on child growth in Ethiopia.

2.3.4 Infection

The infection section factors for Stunting related to both clinical and sub-clinical infection, including enteric infections (e.g. diarrheal disease, environmental enteropathy, helminths), respiratory infections, malaria, reduced appetite from infection and inflammation, in the Amhara Region, reporting that diarrhoea during the 2weeks prior to the study was strongly associated with stunting in children less than 5 years of age (Teshome et al. 2009;Campbell et al. 2003; Humphrey 2009; & Lin et al. 2013)

In general it is know that nutrition is a major risk factor for disease (Bryce et al., 2008). What public-health experts and policymakers have not done is to gather the evidence about the importance of maternal and child nutrition, catalogue the long-term effects of under- nutrition on development and health, identify proven interventions to reduce under nutrition, and call for national and international action to improve nutrition for mothers and children(Reyes et al., 2004).

2.3.5 Socioeconomic and demographic factors

The causes of malnutrition are numerous and multifaceted. These causes are intertwined with each other and are hierarchically related. The most immediate determinants are poor diet and disease which are themselves caused by a set of underlying factors; household food security, maternal/child caring practices and access to health services and healthy environment. These under- lying factors themselves are influenced by the basic factors (Fikadu et al., 2014). The variability in child growth across nations is due much more to social, demographic and economic factors than to genetics(M, Monteiro, Akré, & Clugston, 2015).

2.3.6 Other unknown factors

Even though the problem of stunting in money countries and Ethiopia has been well documented, and several policies and strategies have been taking place for tackling and reducing the prevalence of stunting, still the problem is increased in the country (Abera, Dejene, & Laelago, 2018). This has serious implications that its specific determinants are not clearly understood and other unknown factors are existed(Agency, 2016). Because child health goals for the early part of the next century have specifically targeted improvements in the rates of stunting. World Health Organisation (WHO) in its recently revised "Health for all in the 21st century" has envisaged to reduce the percentage of children under five years who are stunted to be less than 20% in all specific sub-groups within countries by the year 2020 (Wirth et al., 2017). This indicates that there are some unknown factors for the challenging of stunting reduction, as of us the one possible factor will be the difference in altitude factors.

2.3.6.1 Altitude (Elevation) hypoxia

It is estimated that 20 to 30 million people worldwide live at altitudes above 3000 m commonly defined as high altitude; over half of these individuals live in the Andean region of Latin America (Cook, Boy, Flowers, & Daroca, 2005). It is commonly assumed that the prevalence of iron deficiency is higher in vulnerable segments of these populations because of the added iron requirement imposed by the expansion of the red cell mass (Klausen, Rasmussen, Gjellerod, Madsen, & Petersen, 1968). However, the results of anemia surveys to identify this iron deficiency are limited by uncertainty in the criteria of anemia at high altitude (Gonzales, Steenland, & Tapia, 2009).

Altitude exposure increases the body’s need for iron (Cook et al., 2005). Athletes and pregnant mothers are typically recommended to ingest a daily oral iron supplement to facilitate altitude adaptations, and to help maintain iron balance (Garvican-Lewis et al., 2016). Iron is involved in many central nervous system processes that could affect infant behavior and development (Lopez, Cacoub, Macdougall, & Peyrin-Biroulet, 2016). Iron deficiency is the most common form of malnutrition in the world, affecting more than 2 billion people globally (Akhtar et al., 2011). It is highly prevalent in less-developed countries but also remains a problem in developed countries where other forms of malnutrition have already been virtually eliminated (Camaschella, 2017).

However, there is some debate as to whether athletes and pregnant women with otherwise healthy iron stores should be supplemented, due in part to concerns of iron overload. Excess iron in vital organs is associated with an increased risk of a number of conditions including cancer, liver disease, and heart failure and long term mental cognitive effect on the child(Garvican-Lewis, Govus, Peeling, Abbiss, & Gore, 2016).

Also at least half of anemia worldwide is due to iron deficiency, more than 80% of countries in the world, the prevalence of anemia in pregnancy is >20%, and is a major public health problem (Haidar & Pobocik, 2009). More than 56 million women are estimated to be affected globally, approx 32 million of them being from Asia. The global prevalence of anemia in pregnancy is estimated to be approximately 41.8%, varying from a low of 5.7% in the USA to a high of 62.7% in Ethiopia (Goonewardene, Shehata, & Hamad, 2012), which is a severe problem for both pregnant and non-pregnant women of childbearing age (Haidar & Pobocik, 2009)

2.3.6.2 Pregnancy and oxygen impairment on altitudes

The accumulation of oxygen within the atmosphere had two great benefits for life. First, it led to the formation of an ozone layer that acted as a shield against the fierce ultraviolet radiation bombarding the planet. This protection allowed life forms to emerge from the oceans and colonize the land. Secondly, with the exception of fluorine, the reduction of oxygen provides the largest release of free energy per electron transfer(Burton, 2009).

Some study shows that development of chick embryos at high altitude-induced fetal hypoxemia and led to an increase in fetal hematocrit and several studies have reported an increase in packed red cell mass in the umbilical blood of human infants following gestation at high altitude this shows that the partial pressure of oxygen is actually reduced in the fetal blood during development at high altitude (Giussani, Salinas, Villena, Blanco, & Biolog, 2007)

Some previous studies have shown that the incidence of low birth- weight, stillbirth, and neonatal mortality rates are higher at HA in Peru(Tufts Da, 1985). Most populations living at

HA in Peru character is typically have an increase of hemoglobin level due to the effect of hypoxia as a mechanism of compensation. Elevated hemoglobin at HA has also been seen in Tibet (Cohen & Haas, 1999).

Table 1: Adjustments to measured hemoglobin concentrations for smokers

Smoking status	Measured hemoglobin adjustment (g/l)
Non-smoker	0
Smoker (all)	-0.3
½ -1 packet/day	-0.3
1-2 packets/day	-0.5
≥ 2 packets/day	-0.7

Table 2: Classification of public health significance of anemia in populations

Category of public health significance	Prevalence of anemia (%)
Severe	40 or higher Moderate
Moderate	20.0 – 39.9
Mild	5.0 – 19.9
Norma	4.9 or lower

The World Health Organization (WHO) has proposed that hemoglobin values should be adjusted for altitude. With this adjustment, the cutoff hemoglobin value to define anemia increases as altitude increases(Gonzales et al., 2009). There are different cut off for hemoglobin levels depending on the person`s person's age, gender, residential elevation above sea level (altitude), smoking behavior, and different stages of pregnancy, certain diseases and micronutrient deficiencies(WHO, 2014).

2.4 Stunting in Ethiopia

In Ethiopia, the prevalence of under-nutrition manifested in the form of underweight, stunting and wasting among pre-school children (6-59 months) is 45%, 56.7% and 9.6% respectively (CSA, 2000). This implies that the level of protein-energy malnutrition (PEM) is one of the highest in the world and its recent trend.

Stunting in the country also has declined on average by 1.2%per years since 2000. Though the annual rate of stunting reduction in Ethiopia is below the global average, Ethiopia's stunting reduction is noteworthy as it is one of the few African countries to substantially reduce the prevalence and the number of stunted children in the past two decades (Woodruff et al., 2017).

2.5 Role of stunting reduction is not as expected

The global target translates to a 3.9% reduction per year and implies decreasing the number of stunted children from 171 million in 2010 to about 100 million in 2025. However, at current rates of progress, there will be 127 million stunted children by 2025, that is, 27 million more than the target or a reduction of only 26% (De Onis et al., 2013). Despite reductions in the prevalence of stunting globally, stunting remains a serious public health concern in Sub-Saharan Africa where the number of stunted children is rising (Wirth et al., 2017).

Although Ethiopia has made steady progress in reducing stunting shows the trend in the reduction of child under nutrition between 2000 and 2016. The prevalence of stunting has decreased considerably from 58% in 2000 to 38% in 2016, an average decline of more than 1 percentage point per year. On the other hand, the prevalence of wasting changed little over the same time period, with a wasting rate of 10% at the time of the EDHS 2016, which was the same level as in 2011. The prevalence of underweight has consistently decreased from 41% to 24% over the 16-year period (CSA, 2016), this shows that, the prevalence of stunting in the country is become, and it is still one of the highest in the world, and the issue is a national priority in public health (Haile et al., 2016).

3. Chapter Three: Materials & Methods

3.1 Study area

The study was conducted in two different sites one at Debre-Berhan University (DBU) in the laboratory of the Department of Biology (high altitude) and the second at Ziway, Allage Agricultural college (lowland) having different altitudes of Ethiopia

Debre Berhan is a city and woreda in central Ethiopia which is located in the Semien Shewa Zone of the Amhara region, about 120 kilometers northeast of Addis Ababa on the paved highway to Dessie, the town has a latitude and longitude of 9°41′N 39°32′E Coordinates: 9°41′N 39°32′E and an elevation of 2,840 meters above sea level (Local History in Ethiopia, 2012).

Alage ATVET College From the different colleges established under the ministry of agricultural technical vocational education and training college is the one. It is located 215 km south of Addis Ababa around the rift valley lakes bordering the two rift valley lakes abijata and shalla. Alage has an altitude of 1543 meters above sea level (Local History in Ethiopia, 2012). It was established in 1980 as an orphan care center and served under this objective till 1998. In 1998 the institution was converted to rehabilitation center for soldiers victimized during the war with neighboring Eritrea.

The institution attained its present structure and name “Alage agricultural technical vocational educational training college” (Alage ATVET college) in 2002 when the government took the initiative to promote the “national technical vocational education and training” program. Under this program, it was envisaged that the knowledge and skill attained by the trainees specifically in agricultural discipline would fit into the country’s transformation strategy.

Alage ATVET college is established on 4200 ha of land and it possesses infrastructure and facilities most required for practical focused agricultural training the college has four departments namely plant science, animal science, Natural resource and animal health.

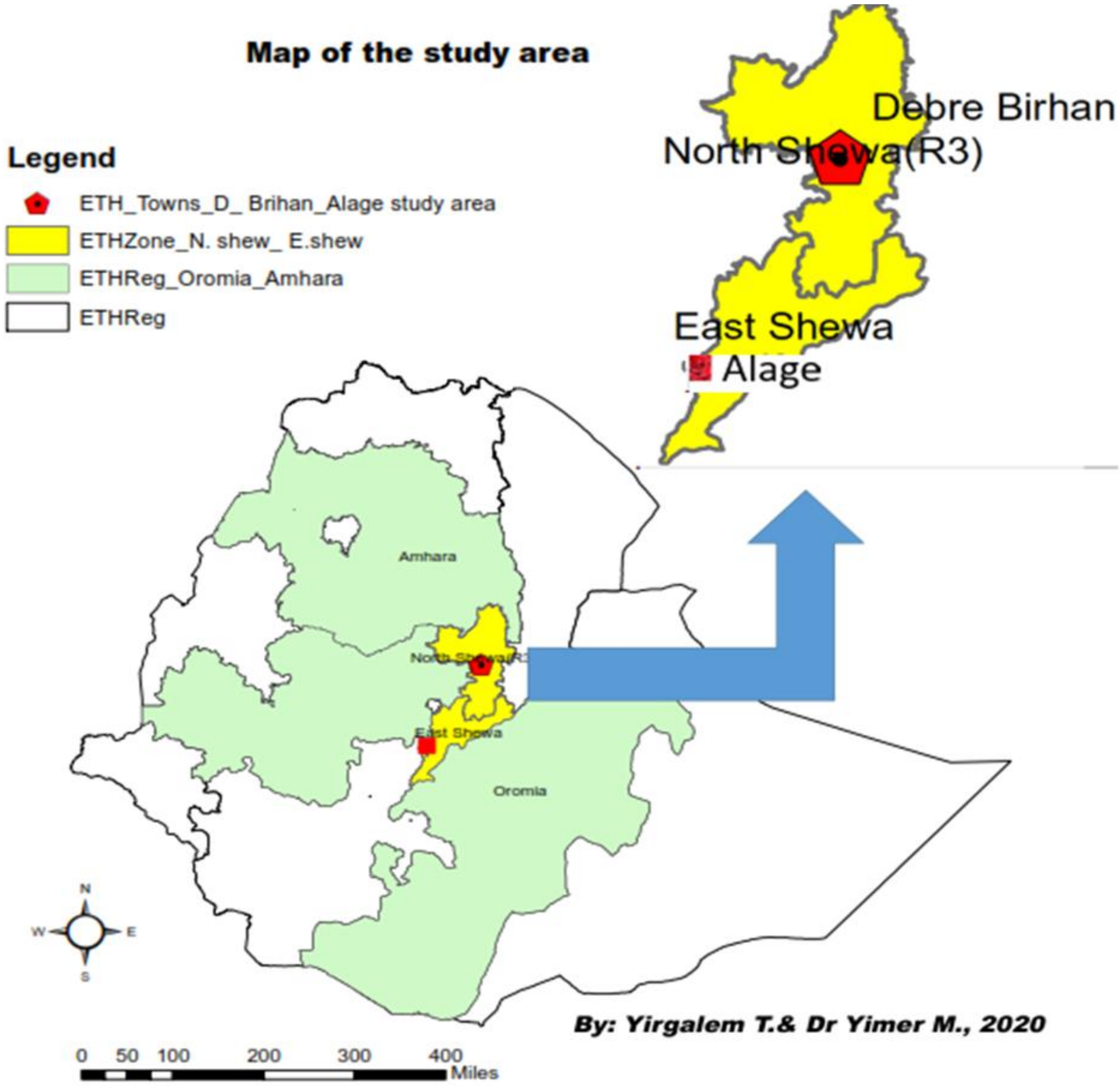

Figure 4: The study sites (area) Dbre-Brihan and Allage

3.2 Ethical consideration

Ethical clearance was obtained from the institutional ethics review board (IRB) of the College of Natural and Computational Sciences, Addis Ababa University.

3.3 Animals

Totally we use 128 wister albino rats (40 for parent, 48 for 1st generation, 40 for 2nd generation) Initially we use female albino wistar rats (n= 32), aged 45 days, an appropriate age to the final stage of puberty, according to Andrade et al., (2002) and weighing 200–250g were met with male albino Wistar rats, 60 days old, (n= 8), weighing 250– 300g we use a total of 40 (in parent experiment 32 female and 8 male rats at the beginning of study that is female are subjected to for High altitude 8 treatment and 8 control, and for Low altitude 8 treatment and 8 control, and 40 male rats were used for mating).

For first generation at high altitude we use a total of 40 female rats for different measuring parameter's that is, for control group we use 8 for Hb test, 8 for MWM, 8 for electric shock test and for control group we use 8 for Hb test, 8 for MWM, 8 for electric shock test and 8 female rats for mating for production of second generation. For first generation low altitude for control group we use 8 for Hb test 8 for MWM, 8 for electric shock test and for treatment groups for control group we use 8 for Hb test 8 for MWM, 8 for electric shock test.

For second generation at high altitude, for control group we use 8 for Hb test 8 for MWM, 8 for electric shock test and for treatment groups for control group we use 8 for Hb test 8 for MWM, 8 for electric shock test. For low altitude for control group we use 8 for Hb test 8 for MWM, 8 for electric shock test and for treatment groups for control group we use 8 for Hb test 8 for MWM, 8 for electric shock test. The rats were obtained from the EPHI Laboratory Animal Facility.

3.4 Transportation

The transportation facility and design of rats to both study site was carried out carefully. The Rats transported by vehicles (Toyota half van) with separate portion by card bored which they can get adequate ventilation the space specified for each animal was broad enough to allow the animal a normal posture and to move freely, avoiding claustrophobic feelings that can cause stress and provide appropriate gripping systems that do not compromise the animal.

We set and put four rats in one standard polypropylene cages (49 cm × 34 cm × 16 cm) with good bedding system and free access to feed but watering is three times at rest time of the journey (because if we provide free water, the water linkage during journey makes the rat's.

Bedding wet or over moisture, which it can be the source of bacteria or disease. The rats were randomized into two study sites groups (High altitude- Debre Berhan and Allage - low altitude) and were housed in polypropylene cages (49 cm × 34 cm × 16 cm). The rats were acclimatized to their environment for 1 week before the start of the experiment, they were subject to a natural photoperiod (approximately 12 h day and 12 h night cycle), and were fed *ad libitum* (Biochemistry, Faculty, & Galim, 1986).

3.5 Experimental design

Female albino wistar rats were randomly allocated to two groups to high and low altitude and further subdivided in the study site to the control group and supplement group with the same number of animals in the two altitude sites. According to the National Academy of Sciences Washington, D.C. 1995, the control group fed on the standard diet which AIN-39G containing (35mg/kg iron diet) and the treatment group was fed on AIN-39G containing IFA supplement (75mg/kg containing iron diet) (Sciences, 1995), until postnatal day 21 for both treatment groups of parents and 1st generation (Felt et al., 2006) and after postnatal day 21 the treatment group continued feeding of AIN-39G containing (35mg/kg iron diet) likewise the control group feed. Female albino wistar rats' mate after 14 days and each experimental group was composed of 8 animals, and two experiments were conducted independently. A total of 32 animals were used in the two altitude sites for the first generation study. After birth, all the pups were kept together with their respective mother and offspring from all groups were weaned 21 days after birth and left undisturbed until testing at 2 months of age (Maccari et al., 1999). Figure 5 presents a schematic representation of the overall experiment.

Note The rats standard Feed was sponsored or denoted by EPHI food science and nutrition department and they were purchased from SAFE scientific animal food and engineering, AIN-39G complete diet for adult rats Route de saint Bris 89290 AUGY, FRANCE. Each packet or sachet containing 75mg/kg (IFA supplement) and 35mg/kg (maintenance face) iron diet separately and it was in the form of coarsely and we prepare the pellet by hand to protect the contamination of iron from iron material.

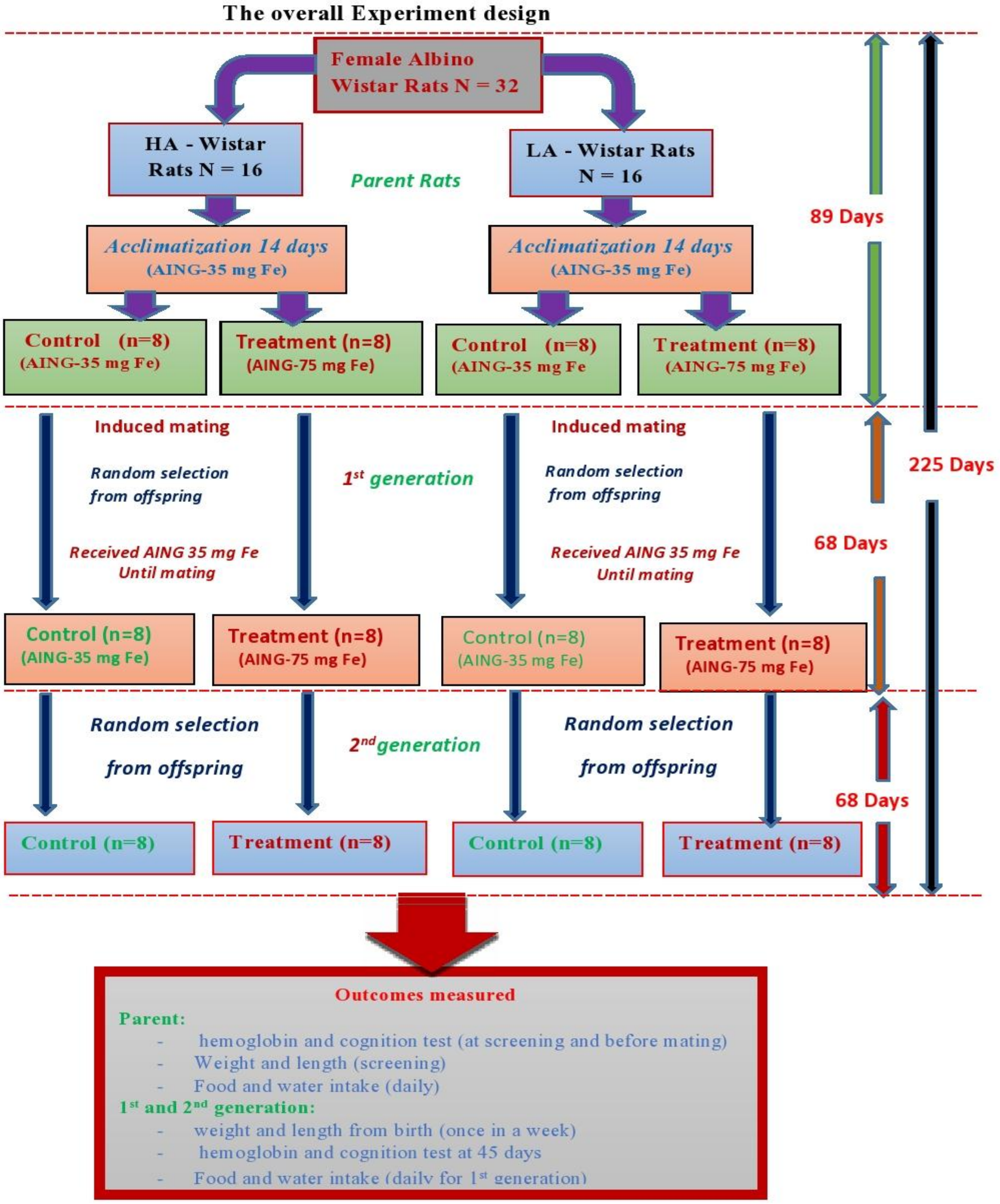

Figure 5 Schematic representation of the experiment

Figure 5: The overall experiment design

Base line easements

The parent rats were screened out by different parameter measured like weight, length, Hemoglobin concentration, CRP, and cognition test in the first 5dates before the beginning of the experiment, and then we randomly allocate each rats to the specified study group with their respective parameters measurement value.

3.6 Weight parameters

Each Rats pup was weighed weekly, and the weight delta defined the difference between final and baseline weights. At the end of the experiment, the naso-anal length (mm) of the animals was measured to determine the linear growth of the Lee index. This index, which was adapted from Dallman, Pecoraro, corresponds to the ratio between the cube root of the body weight (g) and the naso-anal length (cm) of the animals multiplied by 10 (Dallman, Pecoraro, & La Fleur, 2005).

3.7 Blood collection and analysis

Blood was collected from the Lateral saphenous vein of dam rats at baseline and before mating took place for Hb analysis using hemoglobin meter hemocue 301+ (Policies, 2012 & Beeton, Garcia, & Chandy, 2007).

A few drops were used for Hb analysis using hemoglobin meter (hemocue 301+) from randomly selected rat offspring (Macedo et al., 2012). Animal handling euthanasia (in human-animal killing) and all experiments were performed in accordance with the International Guidelines for Animal Welfare (Estrela et al., 2015).

3.8 Behavioral and cognition test

3.8.1 Morris Water Maze (MWM)

The water maze was a round pool of 150cm diameter with 45cm high walls (Neuroscience, Osaka, Japan) surrounded by several extra-maze cues. The extra-maze cues were kept constant throughout the testing period. The tank was filled to a height of 27cm with tap water of approximately 22 ◦C. The transparent escape platform (12 cm in diameter) was placed 1 cm below the surface of the water in the middle of one of the quadrants. The position of the platform was not changed throughout the experiment (Lüttgen et al., 2005). Rats have trained 4 trials per day for 5 days. In each trial, rats were put into the water, facing the pool wall at one of the three starting positions, and then released. If a rat failed to find the platform within 60 s, the rat was manually guided to the platform by the experimenter. Rats were allowed to rest on the platform for 30 seconds after each trial (inter-trial time) and for each trial, and in the fifth day the water opaque by milk powder and the plat form was hidden, and we measured the rats how much time or seconds they were spending to swim to reach to reach and fined the hidden platform, and the time took was recorded (Estrela et al., 2015).

3.8.2 Electric shock for passive avoidance behavior test

The passive avoidance behavior was investigated in a one-trial step-through paradigm (Ader, Weijnen, & Moleman, 1972). The apparatus consisted of a dark compartment (40 × 40 × 40 cm) and a well-lit platform attached to the front side of the dark chamber. A small sliding door separated the two compartments. On the first day of testing, a one min adaptation was allowed in the dark compartment which was then followed by a single trial by placing the animal on the illuminated platform and allowing it to enter the dark chamber. On day 2, after entering the dark chamber an electric foot shock (0.6 mA, 3 s) was delivered (Ader et al., 1972). The measurement of the passive avoidance response was done by the registration of latency in entering the dark compartment 1 day (short-term) and 3 days (long-term) following the electric foot's chock. Avoidance to enter the dark compartment during a 3-min time period was set to 100% and a time-related percentage was given when the animals entered (50% was given to the animal if they entered the chamber after 1.5 min of the start) (Kaneko, Tahara, Nakamoto, & Pucsok, 2001).

3.9 Statistical analysis

Descriptive statistics like mean, median, standard deviation, frequency, and percentages were used. Comparison between treatment and control groups was made using student t-test for parametric and Man–Whitney test for non-parametric data using SPSS software (ver. 20) Package. Repeated measures ANOVA (one- way ANOVA, group effect) was used for repeated measures of MWM test, escape latency and the total swimming distance for 5 days. In all statistical analyses, p-values < 0.05 were considered as statistically significant.

4. Chapter Four: Results and Discussions

4.1 Result

4.1.1 Baseline measurements of parent albino wistar rats

The hemoglobin concentration, body weight, length, and age were similar across the different groups. There was also no significant difference in the behavioral and cognition scores among groups. None of the rats had a positive CRP value. The baseline values suggest that the randomization was effective. (Table: 3).

Table 3: Different parameter value of parent rats at base line measurement

	Treatment `		Control		P-value
	HA	LA	HA	LA	
Weight (g)	238.0 ± 1.77[a]	238.87 ± 1.3a	238.5 ± 1.77[a]	238.62 ± 1.6[a]	0.52
Length (mm)	143.5 ± 3.75[a]	142.62 ±4.13[a]	143.75 ± 2.81[a]	142.62 ± 3.20[a]	0.3
Hemoglobin (g/L)	16.36 ± 0.6[a]	16.30 ± 0. 37[a]	16.53 ± 0.42[a]	16.56 ± 0. 58[a]	0.1
Cognition test (seconds)	37.15 ±1[a]	37.13 ± 0.82[a]	37.08 ± 1.79[a]	37.13 ± 0.56[a]	0.73
CRP*	Negative	Negative	Negative	Negative	

Values are mean ±standard deviation and different superscripts within a row represent a statistically significant difference (P<0.05), Values are mean ± standard deviation and different superscripts within a row represent a statistically significant difference (P<0.05); HA, high altitude; LA, low altitude

Description where, HA = High Altitude, LA = Low Altitude

*CRP test - a test measures the level of C-reactive protein in the body. C-reactive protein levels can be high when there is inflammation or infection in the body or system.

4.1.2 Feed and water intake of parent rats

The parent rats' feed intake showed a significant difference between high and low altitudes (Figure 6). While the feed intake was higher in high compared to lower altitude, the water intake trends was the reverse ($P < 0.05$). This is in line with what is expected and follows patterns adopted at different temperatures. In high altitudes, the temperate is lower and thus favors. Increased food intake for thermogenesis. At lower altitudes, the temperature is higher leading to increased dehydration, which is expected to increase water intake.

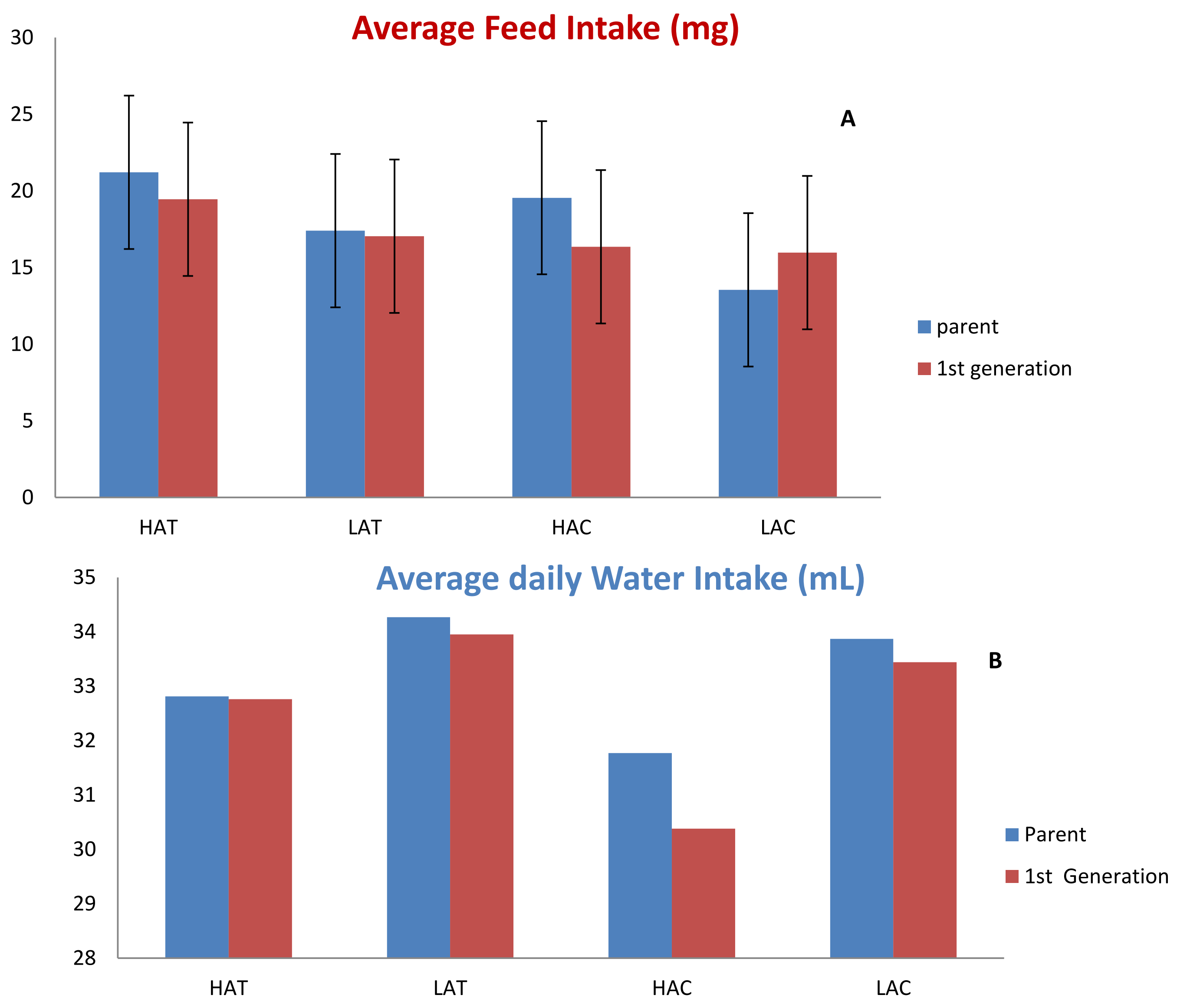

Figure 7: Average daily feed (A) and water intake (B) of parent and 1st generation

HAT = altitude-treatment (75 mg Fe/Kg); LAT = low altitude-treatment (75 mg Fe/Kg);

HAC = high altitude- control (35 mg Fe/Kg); LAC = low altitude-control (35 mg Fe/Kg)

4.1.3 Birth weight, birth length, and mortality

The birth weight of the pups ranged between 4.5 and 7.2 g (figure 7). The lowest birth weight was reported for the high altitude-control group of (1st Generation), whereas the highest birth weight was for the offspring

High altitude-treatment group of 1st generations. Among the control group, the birth weight of the pups was higher for those born at a lower altitude ($P < 0.05$). On the other hand, among the treatment groups, birth weight in high and low altitude was not significantly different ($P > 0.05$). For the first generation pulps, the birth length in the control group was significantly lower at higher than at lower altitude ($P < 0.05$). However, for the treatment group, the birth lengths were equivalent for both high and low altitudes (Fig 8). For the second generation, birth lengths were similar between the high and low altitude in the control and in the treatment groups. However, irrespective of being at low or high altitudes, the birth length was higher in the treatment than in the control group.

The mortality rate of pups was 7 (all 7 of them are only in control groups) in the first generation, and 11(2 from treatment, and 9 of them are from control groups) in the second generation. Mortality was slightly higher at the higher altitude.

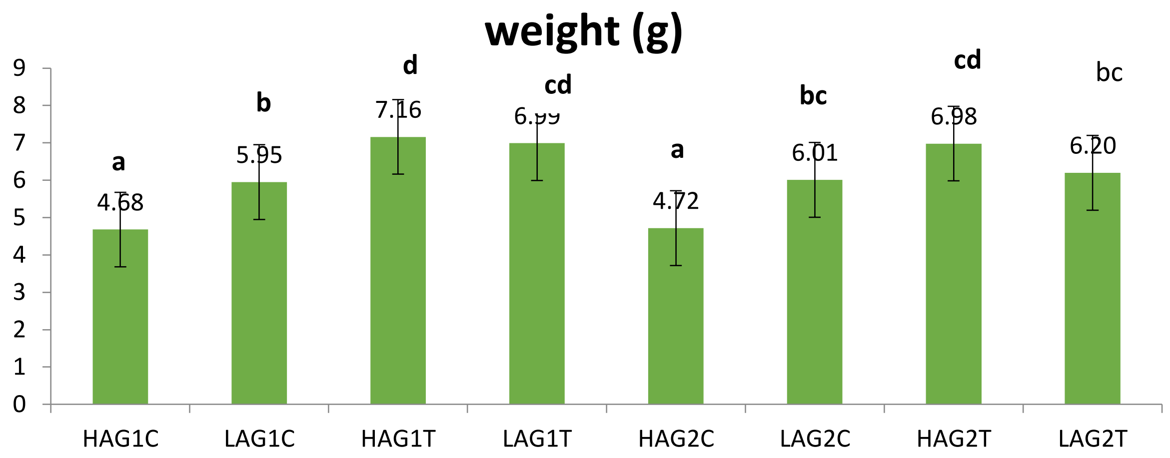

Figure 8: Mean Birth weight (1st Generation & 2nd Generation)

As shown in the figure, statistically, a (HAG1C= 4.6g is relative with HAG2C = 4.7g); b (LAG1C = 5.9g was relatively equivalent of LAG2T = 6.20g); c (LAG1T = 6.99g was relatively equivalent of HAG2T =); bc is nearest value with b and c; and d (HAG1T = 7.1g was not similar with any of them in the group)

Description where, HAG1C = High Altitude 1st Generation Control; LAG1C = Low Altitude 1st Generation Control; HAG1T = High Altitude 1st Generation Treatment; LAG1T = Low Altitude 1st Generation Treatment; HAG2C = High Altitude 2nd Generation Control; LAG2C = Low Altitude 2nd Generation Control; HAG2T = High Altitude 2nd Generation Treatment; LAG2T = Low Altitude 2nd Generation Treatment.

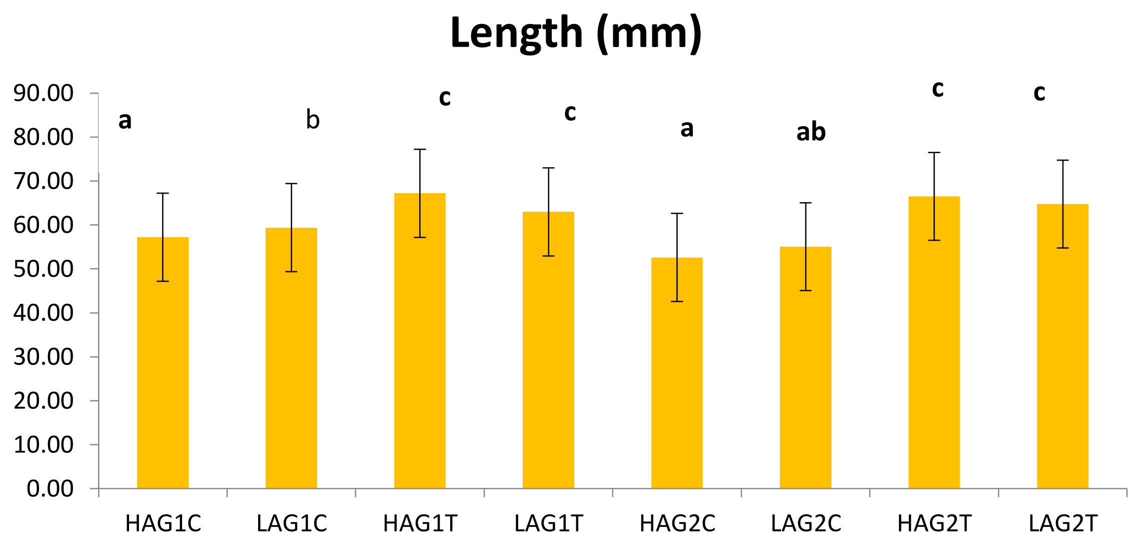

Figure 9: Mean Birth Body Length (1st Generation & 2nd Generation)

Description where, HAG1C = High Altitude 1st Generation Control; LAG1C = Low Altitude 1st Generation Control; HAG1T = High Altitude 1st Generation Treatment; LAG1T = Low Altitude 1st Generation Treatment; HAG2C = High Altitude 2nd Generation Control; LAG2C = Low Altitude 2nd Generation Control; HAG2T = High Altitude 2nd Generation Treatment; LAG2T = Low Altitude 2nd Generation Treatment.

4.1.4 Mean Hemoglobin concentration

In all cases, the hemoglobin concentration of the treatment group was significantly higher than the control group (Fig. 14; P< 0.05). Up to the first generation, there was no difference in hemoglobin concentration between high and low altitude rats found in the same treatment groups. However, in the second generation, a statistically significant different hemoglobin concentration was observed between rats in the control groups of high and low altitude.

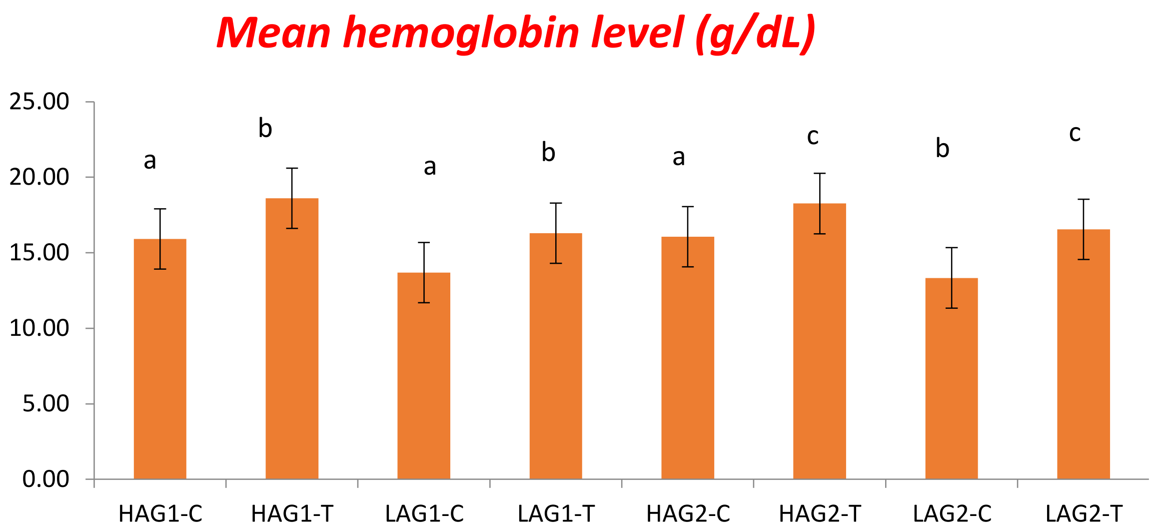

Figure 10: Mean hemoglobin level of 1st and 2nd Generation

Blood sample were taken and analyzed at the age of 45 days old rats. Description where, HAG1C = High Altitude 1st Generation Control; LAG1C = Low Altitude 1st Generation Control; HAG1T = High Altitude 1st Generation Treatment; LAG1T = Low Altitude 1st Generation Treatment; HAG2C = High Altitude 2nd Generation Control; LAG2C = Low Altitude 2nd Generation Control; HAG2T = High Altitude 2nd Generation Treatment; LAG2T = Low Altitude 2nd Generation Treatment

4.1.5 Cognition test

The parent rats' time taken to reach the goal (the plat form) in the 5th date in the water maze experiment was similar (~37 seconds) for all groups (*Table: 5*). However, this was no more the case for the first generation; a significant difference was observed in the time taken to perform the task among rats in the control group (*Figure: 10*) Rats in higher altitude took significantly more time to reach the target than their low altitude counterparts. However, the reverse trend was observed among the treatment groups, where rats in higher altitude took less time to reach the target than their high altitude counterparts.

Table 4: Average probing of parent rats

	Average probing (MWM)			
	HAP-C	**LAP-C**	**HAP-T**	**LAP-T**
Average	37.08	37.13	37.15	37.13
SD	1.79	0.56	1.00	0.82

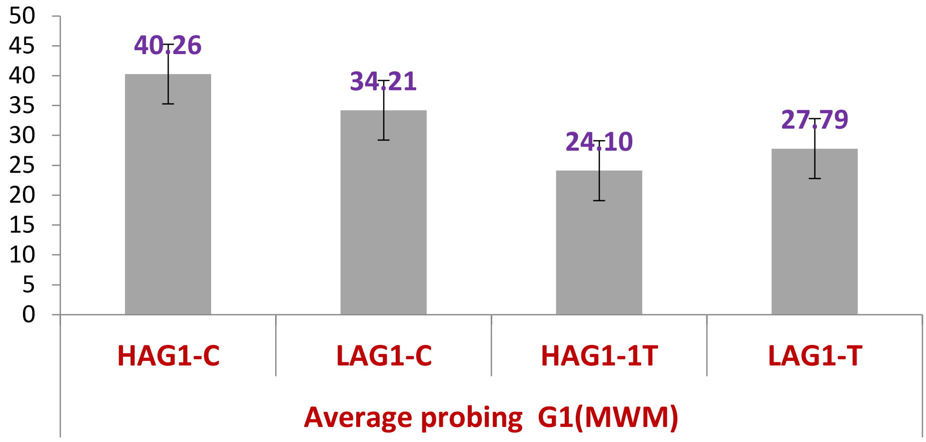

Figure 11 Average probing time (seconds) for the Morries Water Maze test, for first-1st generation

The controls in the second generation took about the same time as the first generations to reach their target (*Figure 12*). The same level of difference was observed between high and low altitude rats in the control group. However, second-generation rats in high altitude treatment groups showed a significantly lower probing time than their lower altitude counterparts. The learning curves of the parents, first and second-generation rats suggested that there was a significantly steeper learning curve for high altitude rats, but this was narrowed when the rats received the treatments (*Figure 13*).

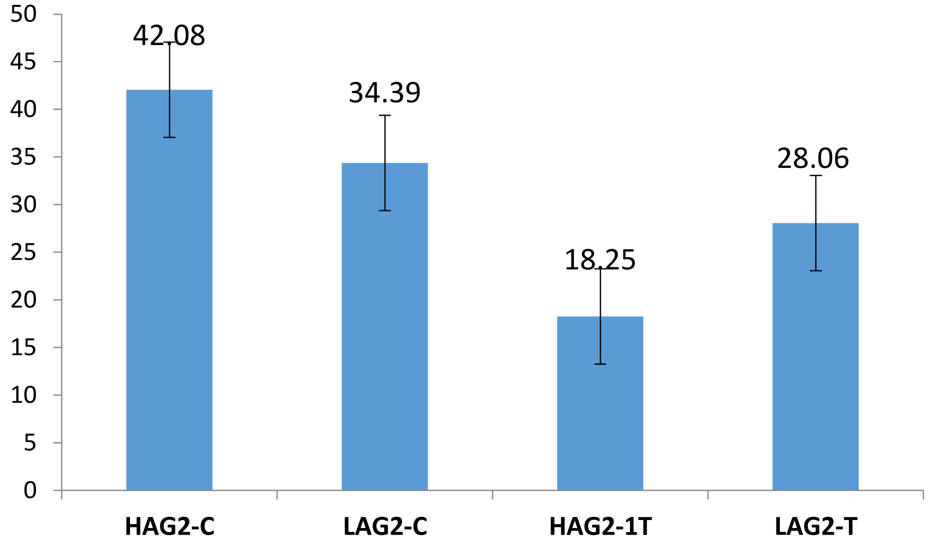

Figure 12: 2nd Generation propping on water maze experiment

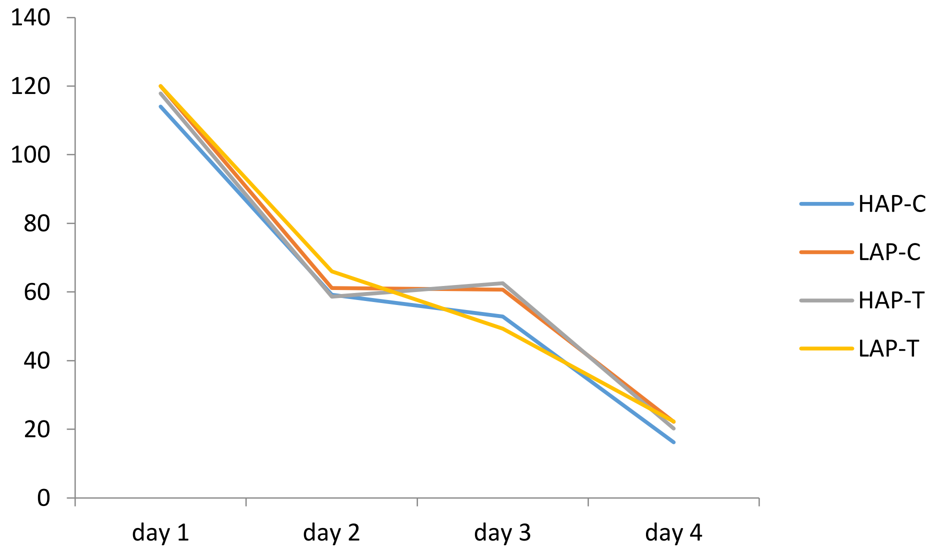

Figure 13: Parent learning curve

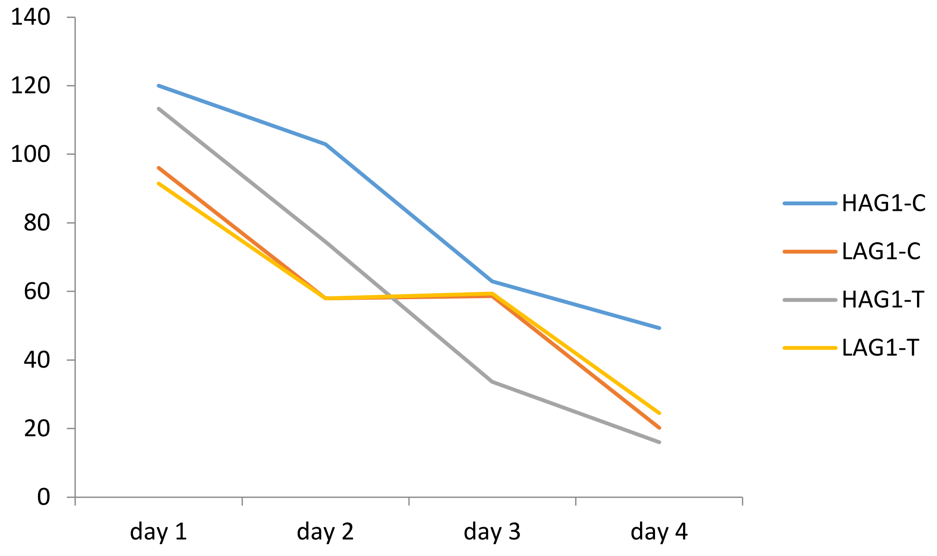

Figure 14: 1st Generation learning curve

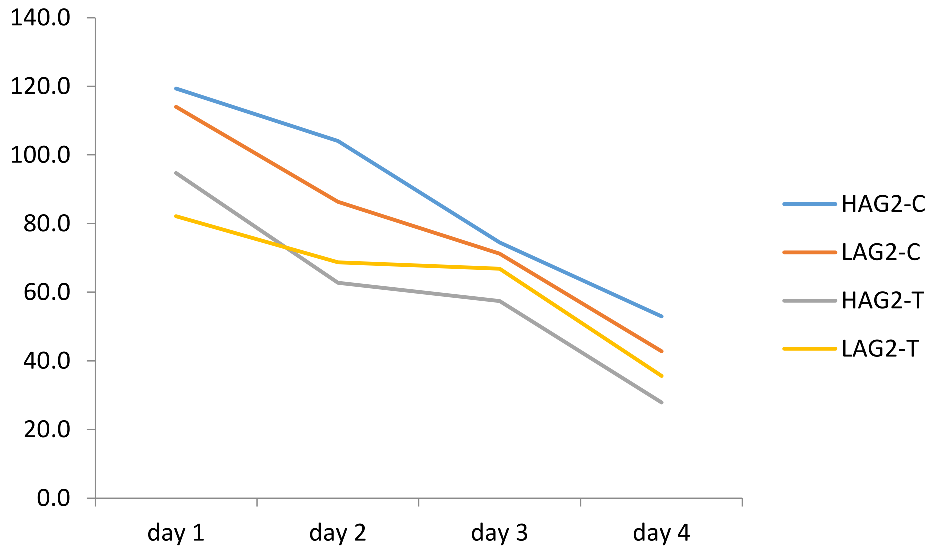

Figure 15: 2nd Generation learning curve

4.1.6 Electric foot shock

The measurement of the passive avoidance response was done by the registration of latency in entering the dark compartment after 3 days (long-term) following the electric foot shock was measured and recorded. As our protocol avoidance to enter the dark compartment during a 3-min time period was set to 100% and, 50% was given to the animal if they entered the chamber after 1.5 min of the start. We give infancies to the long-term memory, so we found the result as following in generation one, treatment group HAG1T= 87.5 ± 23.14 and LAG1T = 87.78 ± 1.4P=1.00. For control groups and in control group HAG1C=75 ±26.72. LAG1C 100 ± 0.00 at p=1.00 and for generation 2, HAG1T= 100 ± 00 and LAG1T = 100 ± 00 =1.00 and HAG1C=75 + 26.7and LAG1C 81.25 ± 25.77at p=1.00.

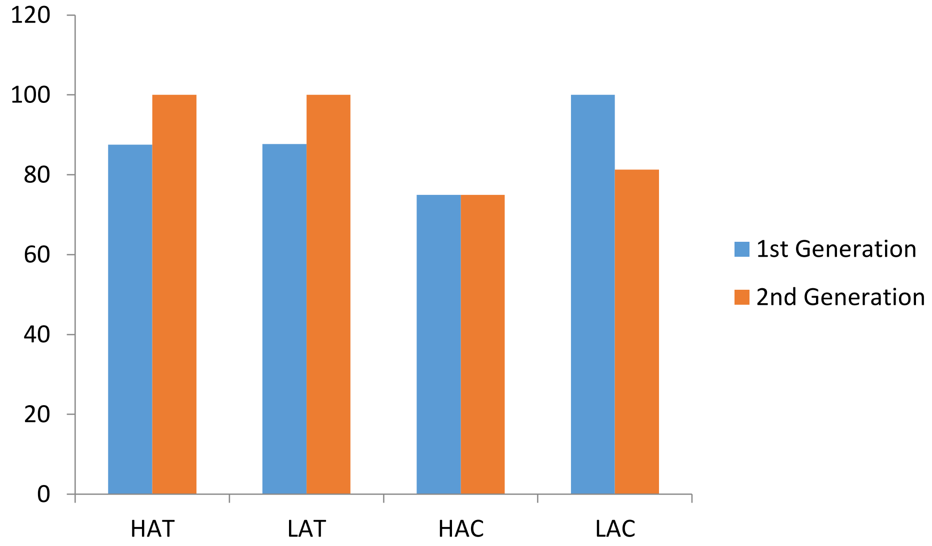

Figure 16: Electric foot shock result in 1st and 2nd Generation

4.1.7 Average linear growth and body weight gain

The linearity growth of curve by weight gain we found that in both rats 1st and 2nd generation the low altitude treatment group (LAT) were the better weight gained and the high altitude treatment groups (HAT) were the second in weight gain, the low altitude control (LAC) groups were in the 3rd passion and high altitude groups were in the least by weight gained throw-out the measurement period up to 60dys old (*Table: 8; 20 & 21*)

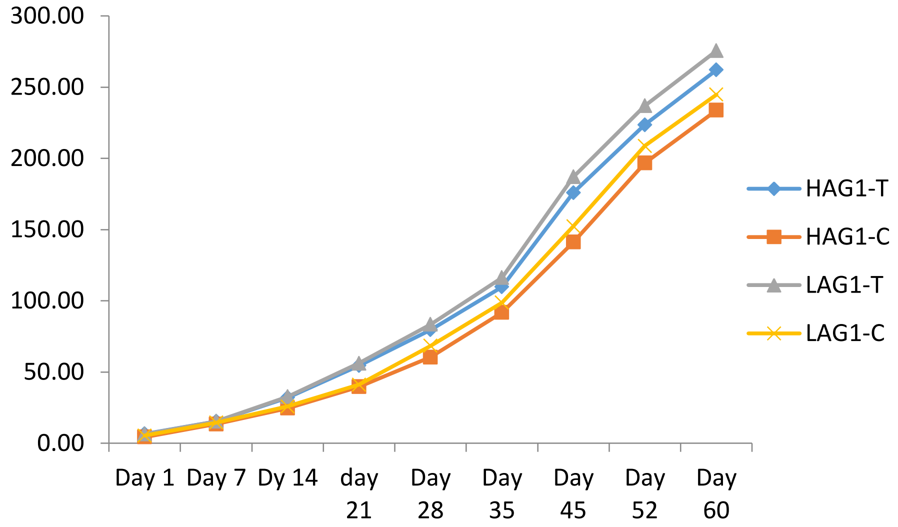

Figure 17: 1st Generations Average Linear growth and body weight gain

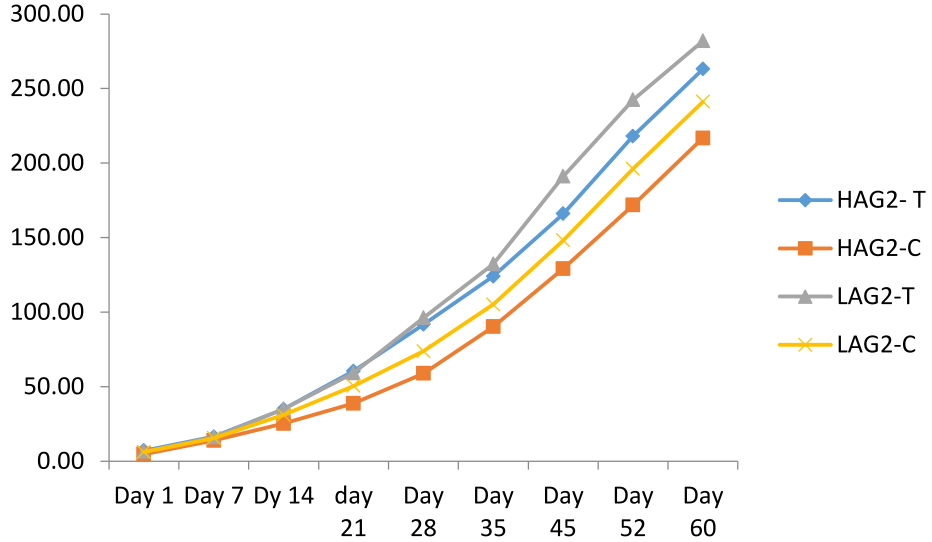

Figure 18: 2st Generations Average Linear growth and body weight gain

4.1.8 Linear growth by body length gain

The linearity growth of curve by body length in both rats 1st and 2nd generation the high altitude (HAT) were the better lengths gained and the low altitude treatment groups (LAT) were the second in weight gained, the low altitude control (LAC) groups were in the 3rd passion and high altitude control groups were in the least by length gained throw-out the measurement period up to 60dys old (*Figure: 19&20*)

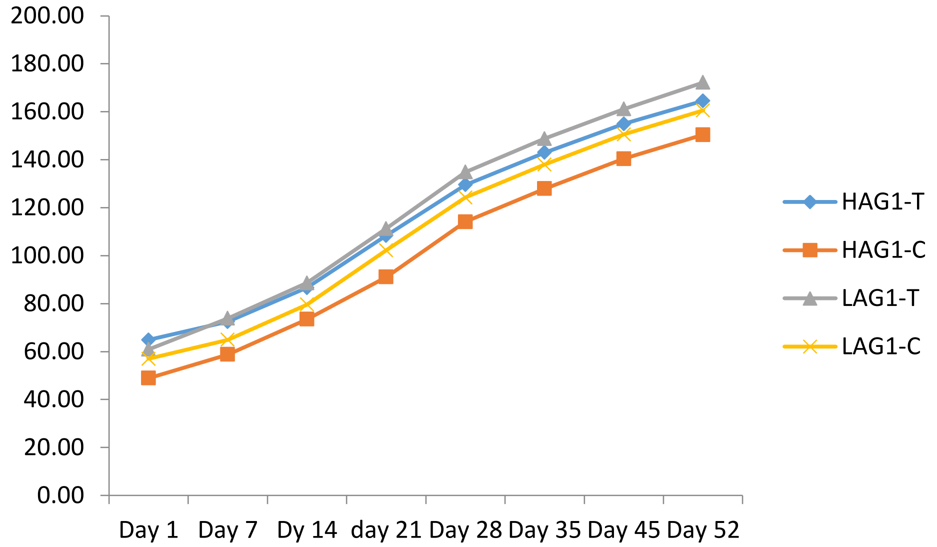

Figure 19: 1st Generation body length growth graph

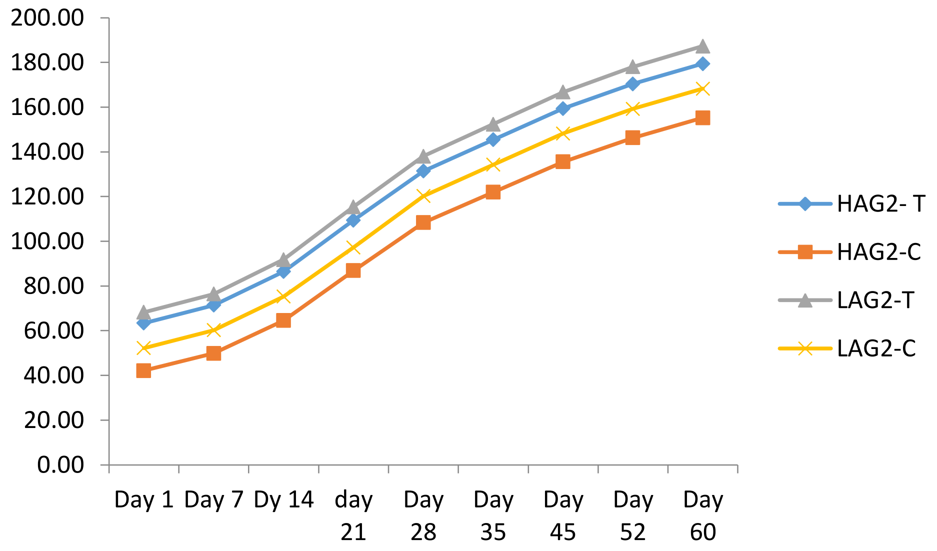

Figure 20: 2st Generation body length growth graph

4.2 Discussions

The study was carried out to determine the Effect of altitude-induced hypoxia in fetal growth restriction and cognitive development in wistar rats. In the comparison of litters birth weight, high birth weight was recorded at high altitude iron treatment groups which was much more greatest than from that of low altitude treatment group by 2.37% in first and by 11.17 % second generation, whereas in the case of comparison of iron treatment and control groups in the same study location (altitude), our result shows that, the HA treatment group was much greater than by 34.64% and 32.38% from that of HA control group and also iron treatment groups of LA increased by 14.88% and 3.06% in both first and second-generation respectively. Between the control groups, LA exceeds the HA by 0.06% and 21.46% in the 1st and 2nd generation respectively. These are findings shows that low birth weight was recorded in the HA control group and the iron treatment group exhibits more benefiters in in getting better litters birth weight in both generation.

Our experimental result is agreed with finding of Jang et al., 2015 those who conduct their experiment in longest duration of hypoxia by administration was from day post conception 7–21 (14 days) with 10% oxygen concentration, was resulted in birth weight reduction by 16.9% ($p < 0.01$) (Jang, Longo, & Goyal, 2015). Furthermore, in a recent study, 12– 14% of oxygen for 7 days (from gestational age 14–21 days) lead to 31% ($P < 0.050$) reduction in pups' birth weight (Deng et al., 2015). Most research pieces of evidence indicate that even a minimum duration of 3 days of hypoxic exposure is necessary for inducing FGR(Jang et al., 2015).

Julian, 2011 and Unger et al., 1988 also stated that the clinical relevance of high-altitude hypoxia for pregnancy outcome is best illustrated by the 3-fold greater incidence of intrauterine growth relative to low altitude. IUGR, defined as restriction (IUGR) at high (2500 m) altitude birth weight less than the 10th percentile of sea-level values for a given gestational age and sex, is associated with

a 4-fold increase in stillbirth and an 8- to 20-fold increase in neonatal mortality depending on the degree of growth restriction(Julian, 2011). Jensen et al., 1997 firmly stated in his finding that as high altitude also reduces birth weight by an average of 120 g per 1000 m elevation gain (Ahankari & LeonardiBee, 2015), (Jensen et al., 1997).

In the case of mean birth length, as we have seen in result section in the first generations there was highly statistically difference between the treatment and the control groups but there was a big change and there was no significant difference between the treatment and the control groups of in 2^{st} generations in that of their birth length outcome. The treatment group of HA in both generation group 67.2mm and 66.49mm was recorded highest than treatment and control groups of LA and

HA but the least mean birth length was recorded in 2^{nd} generation high altitude control group 52.61mm and 1^{st} generation 57.22mm.

The other parameter that we investigate in our experiment was the hemoglobin concentration measurement, and we didn't found any anemic rat groups in all over respective study groups in both generations and also there were no significance difference in mean hemoglobin concentration between groups except between the two control groups in 2^{nd} generation (HAC $=16.0625 \pm 0.713$, LAC $= 13.3375 \pm 0.71$ at ($P < 0.001$) as shown in the Table: 10. Jochmans found the hemoglobin concentration of rat at 14.4–16.0 g/dL at LA and 16.0 - 20.4 g/dL at HA (Jochmans-Lemoine et al., 2015) which is almost similar with our findings

In a lots of science facts shows us that red blood cells contain hemoglobin, which is an essential component of the respiratory system for oxygen transport. Any substantial reduction in red blood cells and hemoglobin reflects a reduced capacity of oxygen transport to tissue. Such a reduction in oxygen transport can be regarded as an adverse health outcome; thus, iron deficiency has a definite effect on health because of anemia (evidence of deficiency)(Yip, 2018).

Jochmans and his friends studied for over 20 years, they have been able to raise laboratory rats (Sprague–Dawley) for more than 30 generations in laboratory conditions at an altitude of 3600 m above sea level, in La Paz, Bolivia and they report that these animals present elevated hemoglobin levels recognizing airspaces in the lungs, and impaired respiratory control (Jochmans-Lemoine et al., 2015).

4.3 Conclusion and recommendations

Iron deficiency is the most common single nutrient disorder in the world, and infants are at particular risk due to their rapid growth and limited dietary sources of iron. Iron is involved in many central nervous system processes that could affect infant behavior and development (Lozoff et al., 2006). Neurotransmitter systems are maturing during key periods of high risk for iron deficiency, and iron is essential for a number of enzymes involved in neuron transmitter synthesis. (Wigglesworth et al., 1988).

Animal studies offer the possibility of studying the effects of iron deficiency that is experimentally induced while controlling for other environmental conditions. They thus play a special role in determining whether iron deficiency causes immediate and long-lasting changes in behavior and development. Animal models also provide direct information about the effects of iron deficiency on the developing central nervous system and thus help interpret some long-term findings in humans. A burst of basic scientific research has provided important new information (Felt et al., 2006).

These studies show not only that iron deficiency during gestation and lactation due to decline or low oxygen concentration at high altitude in the rat results in reduced brain iron, but also that the degree varies greatly by brain region and this research also points to possible mechanisms for long-lasting effects(Beard et al., 2006).

Recommendations

A as recommendation it is better to re-observe the effect of altitude-induced hypoxia on the linear growth of rats, in different parts of Ethiopian altitude by allocating sufficient budget or finance and expertise in the area to develop and prove the gap that we have seen in our experiment before conducting to studying in human which takes a very long period of time budget budget.

This study will be highly valuable and will be an icon indicator on iron supplementation and its impact and effect in different for researchers, donors, policymakers and for the government to prove maternal health and child development of the nations.

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Annex

Annex I: Ethical clearance from the Collage of Natural Science Institutional Review Board (CNS-IRB)

COLLEGE OF NATURAL & COMPUTATIONAL SCIENCES
Addis Ababa University

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OFFICE OF THE DEAN
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Ref. No.
ቁጥር CNSDO/185/12/19
Date:
ቀን፡ November 13, 2019

To Whom It may Concern

The College of Natural & Computational Science Institutional Review Board (CNS-IRB) Committee in its meeting held on October 18 /10/2019 Minute No. IRB/40/219 has examined the project proposal entitled **"Effect of altitude-induced hypoxia in fetal growth restriction and cognitive development in Wistar rats , by Yergalem Tadesse (MSC –Regular) from the Addis Ababa University.by Gebre Yohans Ybelh** from the Addis Ababa University.

The proposal is approved for implementation.

With regards,

Dr. Addisalem Abathun
Dean , College of Natural & Computational Science

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Annex II: Parent feed, water intake, the numbers of litters they give birth and mortality rates

Class	Treatment			Control		
1. Parent	HA	LA	P. value	HA	LA	P. value
Feed Intake	21.21 ± 1.20	17.41 ± 0.38	0.00	19.55 ± 1.23	13.54 ± 0.62	0.00
Water Intake	32.81 ± 0.34	34.27 ± 0.38	0.001	31.77 ± 0.56	33.87 ± 0.60	0.00
No of litters	45	54		58	38	
Mortality %	4(8.8%)	0		3(5.17%)	0	
Mean birth weight	7.16 ± 0.50	6.99 ± 0.46	0.001	4.68 ± 0.73	5.95± 0.99	0.00
Mean birth length	67.20 ± 1.71	62.98 ± 2.97	0.00	57.22 ± 1.43	59.39 ± 3.82	0.00
1st Generation						
Feed Intake	19.45 ± 0.44	17.04 ± 0.58	1.00	16.35 ± 0.9	15.97 ± 0.83	1.00
Water Intake	32.76 ± 0.88	33.95 ± 0.87	0.00	30.38 ± 0.47	33.44 ± 0.85	1.00
No of litters	49	42		38	39	
Mortality %	4(8.16%)	4(9.52%)		2(5.26%)	1(2.56%)	
Mean birth weight	6.98± 0.38	6.20±0.91	0.00	4.72±0.76	6.01± 0.91	0.00
Mean birth length	66.49 ± 2.83	64.74 ± 1.86	0.634	52.61± 0.92	55.08±1.83	1.00
Mean Hemoglobin(g/dL) level at 45 day	18.61 ±1.35	16.3 ± 0.36	0.25	15.91 ± 0.519	13.68 ± 0.72	0.26
Cognition (MWM)	24.1 ± 1.59	27.78 ± 1.41	0.23	40.26 ± 2.10	34.21 ± 2.3	0.20
Cognition Electric shock	87.5 ± 23.14	87.78 ±1.4	1.00	75 ±26.72	100 ± 0.00	1.00
2nd Generation						
Mean Hemoglobin(g/dL) level at 45 day	18.26 ±1.828	16.55 ± 0.72	0.28	16.0625 ± 0.713	13.33 ± 0.71	0.00
Cognition (MWM)	18.25 ± 2.98	28.06 ± 2.9	0.25	42.075±1.2	34.38 ± 4.80	0.23
Cognition Electric shock	100 ± 00	100 ± 00	1.00	75 + 26.7	81.25 ± 25.77	1.00

Annex III: Parent learning

	Day 1	Day 2	Day 3	Day 4
HAP-C	114	59.15	52.8	16.18
LAP-C	120	61.15	60.66	22.17
HAP-T	117.8	58.63	62.56	20.21
LAP-T	120	66.025	49.26	22.23

Annex IV: 1st Generation learning curve (A) probing (MWM) (B)

A)

	Day 1	Day 2	Day 3	Day 4
HAG1-C	120	102.95	62.92	49.325
LAG1-C	96.025	58.025	58.63	20.23
HAG1-T	113.25	74.525	33.68	16.05
LAG1-T	91.425	57.9375	59.4	24.51

B)

	Average probing G1(MWM)			
	HAG1-C	**LAG1-C**	**HAG1-T**	**LAG1-T**
Average	40.2625	34.2125	24.1	27.7875
SD	2.10	2.34	1.59	1.41

Annex V: 2nd Generation learning curve (A) probing (MWM) (B)

A)

	Day 1	Day 2	Day 3	Day 4
HAG2-C	119.4	104.1	74.5	53.0
LAG2-C	114.1	96.7	69.3	42.8
HAG2-T	94.8	62.7	55.7	27.9
LAG2-T	82.1	68.7	66.8	35.6

B)

	HAG2-C	LAG2-C	HAG2-T	LAG2-T
G2Average propping	42.075	34.3875	18.25	28.0625
SD	1.29	1.29	2.98	2.96

Annex VI: Average Leaner growth and body weight gain

	Day 1	Day 7	Day 14	day 21	Day 28	Day 35	Day 45	Day 52	Day 60
HAG1-T	6.64	15.34	32.01	54.49	79.71	109.82	175.99	223.63	262.08
HAG1-C	4.42	13.47	24.6	39.62	60.49	91.79	141.29	196.79	233.79
LAG1-T	6.36	15.4	32.74	56.21	83.5	116.25	187.09	236.91	275.59
LAG1-C	5.43	14.48	25.83	41.18	68.38	98.81	152.31	208.81	244.81
HAG2- T	7	16.4	34.9	60.4	91.7	124	166	218	263
HAG2-C	4.69	13.85	25.23	38.69	58.74	90.19	129.14	171.72	216.69
LAG2-T	6.37	16.02	35.01	59.12	96.12	132.27	191.11	242.42	281.88
LAG2-C	6.04	15.44	30.94	50.44	73.74	105.12	148.12	196.12	241.12

Annex VII: Leaner growth by body length gain

	Day 1	Day 7	Day 14	day 21	Day 28	Day 35	Day 45	Day 52	Day 60
HAG1-T	64.83	72.44	86.58	108.33	129.51	142.98	154.96	164.56	172.22
HAG1-C	48.87	58.77	73.47	91.07	114.07	127.87	140.37	150.37	158.37
LAG1-T	60.95	73.83	88.58	111.28	134.86	148.78	161.15	172.19	179.42
LAG1-C	57.00	64.90	79.60	102.20	124.20	138.00	150.50	160.50	168.50
HAG2-T	63.41	71.41	86.41	109.41	131.41	145.41	159.41	170.41	179.41
HAG2-C	42.11	49.89	64.50	86.89	108.32	121.95	135.58	146.29	155.21
LAG2-T	68.23	76.44	91.84	115.44	138.02	152.39	166.76	178.05	187.29
LAG2-C	52.24	60.24	75.24	97.24	120.24	134.24	148.24	159.24	168.24

Annex VIII: Leaner growth by body length gain

	Day 1	Day 7	Day 14	day 21	Day 28	Day 35	Day 45	Day 52	Day 60
HAG1-T	64.83	72.44	86.58	108.33	129.51	142.98	154.96	164.56	172.22
HAG1-C	48.87	58.77	73.47	91.07	114.07	127.87	140.37	150.37	158.37
LAG1-T	60.95	73.83	88.58	111.28	134.86	148.78	161.15	172.19	179.42
LAG1-C	57.00	64.90	79.60	102.20	124.20	138.00	150.50	160.50	168.50
HAG2- T	63.41	71.41	86.41	109.41	131.41	145.41	159.41	170.41	179.41
HAG2-C	42.11	49.89	64.50	86.89	108.32	121.95	135.58	146.29	155.21
LAG2-T	68.23	76.44	91.84	115.44	138.02	152.39	166.76	178.05	187.29
LAG2-C	52.24	60.24	75.24	97.24	120.24	134.24	148.24	159.24	168.24

Annex IX: Figures of some activities at works before and after experiment done.

Preliminary works like constructing metal mesh cages For the protection predator (wiled Mice & Rats) MWM ,electric foot shook, feed preparation and screening of parent rats by measuring different parameters (body weight, length, hemoglobin cognition)




Feed (pellet) preparation by hand from the standated AIN-39G powder by hand



Journey (traveling) to study sites



Settling the parent rats in the study site

Working in lab