

# Comparative Study Depending on Immune and Body Status with Anemia in Sheep

Inam A. Nayyef

Department of Animal Production, College of Agricultural Engineering Sciences, University of Baghdad, Iraq  
Email: [inamabdelwahed2018@gmail.com](mailto:inamabdelwahed2018@gmail.com)

## ABSTRACT

Current study was reported to indicate effect of anemia on leukocytes concentrations and serum proteins furthermore correlation between anemia and body status with some variables which refer to animals, comparative was depended on anemia and body status, a significant differences were for anemia among various immune variables except basophils however, neutrophils raised to 55.11% for anemic weaker animals while reduced to 44% in healthy moderated body which had higher percentage of lymphocyte, monocyte and eosinophils were 47.40%, 6.20% and 2.00% respectively, on other hand, albumin, globulin and total protein tend to upward for anemic weaker animals reached to 3.51 mg/dl, 4.84 mg/dl and 8.39 mg/dl compared with healthy individuals were received to 3.37 mg/dl, 4.00 mg/dl and 7.39 mg/dl correspondingly. Correlations appeared were insignificantly between each of anemia and body status with studied factors also negative between body status and both of birth type - 0.079 so as sex - 0.220 and adults 0.398 as well as anemia with young lambs - 0.238, association between anemia and body condition was significantly negative - 0.647, Interleukin 12 (IL-12) not affected by anemia, correlation between them reached to 0.127.

**Keywords:** Sheep, anemia, proteins, serum, immunity

## Correspondence:

Inam A. Nayyef  
Department of Animal Production, College of Agricultural Engineering Sciences,  
University of Baghdad, Iraq  
Email: [inamabdelwahed2018@gmail.com](mailto:inamabdelwahed2018@gmail.com)

## INTRODUCTION

Field of animal Production facing main problems around the world occurred in ruminants particularly in sheep which have economic importance for wool, milk and meat production (Raoof *et al.*, 2017). Anemia is the most concern issue which defined basically a reduction of red blood cells that transfer oxygen with insufficient rates for body necessity and physiological requirements also unadequacy of oxygen transferred by Hemoglobin which reduced because of deficiency of iron that entered as a basic component for heme group of hemoglobin in addition, immature RBC in bone marrow stop to division so that, enzymatic action affected and change receptor expression on RBC that stay too long time unreleased from bone marrow furthermore, microphages in marrow declined erythroid progenitors that regulated via erythroblast macrophage protein (Grimes and Fry, 2015), levels of hemoglobin different according to sex and age (Chaparvo and Suchdor, 2019), commonly anemia occurs during gestation period affect neonatal weight and early embryonic mortality in ruminants (Musk *et al.*, 2019) also regression in daily weight unavailability low physiological action muscles weakness and mortality in the acute cases (Gi *et al.*, 2018), less hemoglobin concentration perform to hypoxia which correlated with incomplete births and abortion (Musk *et al.*, 2017). Anemia symptoms known throughout white internal membrane of lower eyelid, yellow skin and in a chronic case appeared bottle jaw moreover, negative effect on performance and production (Griffler, 2020), decreased production of erythropoiesis (Hamdi, 2018) which resulting diminution life span of erythrocytes in addition, bone marrow responding will be down ward so that, hypoxia presence in erythropoietin then produced cells with various rates thus, RBC take several days for maturing after that releasing in circular system (Weiss *et al.*, 2020), on other side, anemia may be products via deficiency of some minerals associated with heinz bodies formation and their relation with oxidative decay on erythrocytes

however, vitamin B12 is important for enzymatic regulation and white blood cells synthesis (Katsogiannnou *et al.*, 2018), anemia happened in adults popularly as well as postpartum ewes on other hand, available of endo and ecto parasites marked up anemia as a result of iron lock in body (Singh *et al.*, 2014) that conformed 60% of hemoglobin which involves heme and contributed in synthesis of several proteins be responsible on oxygen translation also generation of required energy for cells (Grotto, 2008), neutrophil have low action for myelo peroxidase enzyme which produces active oxygen then moderated the responsible for setting of pathogens (Ekiz *et al.*, 2005). Interleukin 12 linked moderately with erythrocytes (Nayyef, 2018), cytokine interfere in down regulation to permit heme synthesis and TNF  $\alpha$  too interacting some interleukins with the signals during erythropoietin receptor and down-regulation by interferon  $\gamma$  so, furthermore, TNF $\alpha$  with free radicals can reduce life span of damaged RBC by linking antibodies with RBC surface which eaten up through phagocytosis (Grimes and Fry, 2015). For serious effects of aneme on health and animal production so as, relation with immunity and body tolerance thus, this research was detected to highlight immunity, body status and their association with anemia in Awassi sheep.

## MATERIAL AND METHODS

Present study located in sheep Farm that refers to College of Agricultural Engineering Sciences, University of Baghdad for period between 3/9/2020 - 15/12/2020 was included 40 Awassi sheep with different ages were divided into two groups depending on health and body status, the first one contained anemic waked sheep while the second group was healthy without anemia moderated body, veterinarian records were considered for health evaluation in the same time visually checking and body mass assumed to determine body status, animals fed on green forage freely with concentrated intake twice a day

intra open barns and no grazing also scattering pesticides to avoid parasitosis.

#### Serological analyses

Blood samples were prepared from congenital vein then divided to two groups, the first put in tubes with anti-agglutination EDTA after that taken to laboratory for counting differentiate leukocytes by using covering glass slides with smears fresh blood Leshman via microscope, other blood samples sited in vacutainers empty of anti-agglutination which clotted and centrifuged at 2500 r.p.m for 7 minutes to produce serum then frozen at -20°C until time of the analysis assay however, total protein was conducted by biuret colorimetric method ( Kaplan and Szalbo, 1983 ), estimation of albumin was throughout Bromo cresol green way ( Johnson, 2008 ), globulin was produced from deducting albumin from total protein ( Scimone and Rothstein, 2012 ).

#### Statistical analyses

Least Square means were calculated for each of white blood cells and serum proteins by using SAS (2012) according to the next models:

$$Y_{ijklmn} = \mu + E_i + M_j + O_k + S_l + P_m + e_{ijklmn}$$

$\mu$ : general mean,  $E_i$ : lymphocyte,  $M_j$ : neutrophil

$O_k$ : monocyte,  $S_l$ : eosinophils,  $P_m$ : basophils

$$Y_{ijkl} = \mu + T_i + B_j + U_k + e_{ijkl}$$

$T_i$ : albumin  $B_j$ : globulin  $U_k$ : total protein

Linkage coefficient was calculated between factors refer to animals: birth types, sex and age which included double groups, the first lambs from birth to yearling while the other was contained adults with age more than one year with each of anemia and body status (Patterson and Thompson, 1971), relationship between anemia and IL - I2 was considered

## RESULTS AND DISCUSSION

### Effect of anemia on immunity

Lymphocyte rate received to 47.40% for healthy moderated while decreased to 33.88% for anemic weakened animals which influenced significantly (table 1), reduction of lymphocyte may be due to negative effect of anemia on immune response amplitude and evolution in lymphocyte expansion then decreased as a reaction to different mitogens which influenced by infection. These results agree with findings of Ekiz *et al* (2005) f about reduction of lymphocyte which coupled with anemia. Neutrophils significantly different between both of anemic waked and healthy moderated animals were 55.11% and 44.00% respectively (table 1) when increased neutrophils were noticed in serum of anemic sheep in study of katsogiannou *et al* (2018), rising neutrophils in current paper perhaps throughout exposing waked sheep to Pathogens that act on multiply phagocytes and phagocytosis (Highland, 2019).

Monocyte at anemic waked sheep highly influenced by anemia was 5.22% whereas tended upward to 6.20% belong to anemic moderated animals ( table 1 ), these values constricted with concentration of monocyte which reached to 7.7 mg/IU against 3.12 mg/IU for anemic and healthy subjects sequencely (kalechman *et al*, 1994).However, modern study correspondent with outcome of katsogiannou *et al* (2018 ) whom indicated to reduction of monocyte perhaps because of iron deficiency which has a fundamental role in the differentiate of monocyte perform to defect in their confirmation process which negatively influence on proteins expression.

Anemic waked individuals have ratio of eosinophils significantly lower than those for healthy moderated reached to 1.59% and 2.00% seriously (table 1), the

reason of eosinophils fall off probably back to inadequate enzymes that contributed to form these cells inter bone marrow as a result of exposing to additive stress ( Gondahi, 2019 ). Percentages of basophils were 0.10% and 0.51% for both of anemic weakened and non-anemic moderated animals respectively which not influenced by anemia (Table 1). This result proved that there is no stimulation factor for these cytes to generate allergy in the case of anemia.

### Anemia and some of serum proteins

Evaluation of albumin for anemic weakened sheep received to 3.51 mg/dl while minimized to 3.37 mg/dl belong to healthy moderated individuals ( $p < 0.01$ ) (table 1), this study a crossed with the results of Laughran *et al* (2017) a round reduction of albumin which coupled with anemia, on the same side, current values were higher compared with those for anemic 2.52 mg/dl and 3.07 mg/dl due to healthy sheep ( AL-Hadithy and Baldawi, 2015), the cause of raising albumin at anemic weakened sheep may be refer to their infection with inflammations at the same time with anemia when causing malfunction of body fluids that required secretion over amounts of albumin to protect tissues from damage.

Approximated globulin values for each of anemic weakened and healthy moderated animals significantly were 4.84 mg/dl and 4.00 mg/dl correspondingly (table 1 ) disagree with this result, higher concentration was 3.05 mg/dl for healthy then declined to 2.73mg/dl due to anemic group ( AL-Hadithy and Baldawi, 2015 ), also reversible to listings of kancko *et al* (2008) whom reported less concentration of globulin at anemic animals, the reason of boosting globulin in modern output may be go to disturbances in immunity system because of anemia which stimulate the body to produce antibodies perform to red blood cells decay.

Total protein significantly different and affected by anemia and body status was 8.39 mg/dl for anemic waked whereas lacked to 7.39 mg/dl in healthy moderated subjects (table 1). This returns inversion with consequences of Al-Hadithy and Baldawi (2015) that is total protein valued 5.25 mg/dl for anemic and increased to 6.13 mg/dl at healthy sheep, on the same side, current findings were the same direction according to significance with results of Radostitis *et al* (2007) as well as varied with Javed *et al* (2010), increasing of total protein probably refer to raising proteins concentrations in serum.

### Correlations

Association between anemia and body status recorded negative with high significant was - 0.647, on the other hand insignificant linkage between anemia and each of birth type, sex, lamb and adults received to 0.051, 0.235, - 0.238 and - 0.398 respectively so as, relation between body status with birth type - 0.079 sex - 0.220 and adults - 0.323 however, positive with lambs 0.165 (table 2) this indicated that when body status is well coupled with diminution cases of anemia, this reflects increasing of body resistance with excess body mass, present results came fit to output for singh *et al* (2014) whom membered that anemia occurred in young animals more than adults because of their rapid growth which demand large amounts of iron to produce hemoglobin, many of previous studies were reviewed importance relation between male and female with anemia ( AL-Hadithy and Baldawi, 2015), whilst Loughran *et al* (2017 ) were reported insignificant variance between single and twin births with anemia, however, Casanova *et al* (2018) said that anemia occurrence depending on sex and age, on the

other hand, ineffective positive relation found between anemia and interleukin 12 tended to 0.127 (table 2), another study for kalechman (1994) contradict to this finding which noticed a key association between anemia and cytokines levels throughout effect of hemoglobin changes on cytokin secretion among anemic and healthy individuals, IL-12 helps to activate and regulates immune cells by promoting T helper cells and activate mediated immune cutes by high affinity for two sub units which

secreted natural immunity ( Lee *et al*, 2014 ) any way, IL-12 displayed from eosinophils and when these cells reduced with occurrence of anemia therefore, IL-12 not influenced by this case and secreted indifference amounts. Eosinophils estimated by Th1 and Th2, cells (Foti & Locati, 2017), this means that T helper cells not recruited at anemia, this explains slight relationship between IL – 12 and this case.

**Table 1:** Least square means  $\pm$  standard error for white blood cells and serum proteins according to health and body status

Variables	Anemic weaked $\pm$ Standard error n = 20	Healthy moderated $\pm$ Standard error n = 20
Lymphocyte %	33.88 $\pm$ 5.80 b	47.40 $\pm$ 3.54 a **
Neutrophils %	55.11 $\pm$ 4.79a	44.00 $\pm$ 4.39 b **
Monocyte %	5.22 $\pm$ 0.95 b	6.20 $\pm$ 1.59 a **
Eosinophils %	1.55 $\pm$ 0.62 b	2.00 $\pm$ 0.70 a. **
Basophils %	0.10 $\pm$ 0.10 a	0,57 $\pm$ 0.36 a. NS
Albumin mg/dl	3.51 $\pm$ 0.47 a	3.37 $\pm$ 0.40 b. **
Globulin mg/dl	4.84 $\pm$ 1.07 a	4.00 $\pm$ 0.42 b. **
Total protein mg/dl	8.39 $\pm$ 0.76 a	7.39 $\pm$ 0.25 b. **

\*\* (P<0.01) NS: not significant

Means with the same letters are not different between each of other

**Table 2:** Correlation coefficient between studied variables with each of anemia and body status

Variables	Anemia	Body status
Birth type	0.51 NS	- 0.079 NS
Sex	0.235 NS	- 0.220 NS
Lamb	- 0.238 NS	0.165 NS
Adults	0.398 NS	- 0.323 NS
IL – 12	0.127 NS	
Body status	- 0.647 **	

## CONCLUSION

Reliance on current listings, conclusion that white blood cells increased at healthy moderated animals and this parameter to their high immunity and resistance so that proved by significant negative linkage between anemia and body condition when proteins rising in anemic waked animals as a result to raising of body requirements to proteins for compensation and maintain of body viability.

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