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# Determining the reference values for arterial blood gas, electrolytes and acid-base in healthy male sheep of Iranian Shall breed

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## Abstract

The goal of this research was to determine the normal range of arterial blood gas, electrolytes and acid-base status in the healthy male sheep of Iranian Shall breed. For this purpose, the blood sample was collected from the ear artery of 15 Iranian Shall sheep whose health was confirmed by clinical examinations, hematological tests including complete blood count and air-dried blood smear and cardiac evaluations including electrocardiography and echocardiography. Then, the values of pH, partial dioxide carbon pressure, partial oxygen pressure, oxygen saturation, base excess, bicarbonate as well as the concentration of electrolytes and anion gap were measured using a blood gas analyzer. The obtained data were subjected to statistical analyses by SPSS software and the tests of Kolmogorov-Smirnov and Pearson and descriptive statistics of the results were expressed as mean ± SE, range (minimum and maximum) and confidence interval 95.00%. The results showed the mean value of arterial blood gas including oxygen saturation, partial oxygen pressure, and partial dioxide carbon pressure were 95.69%, 61.58, and 26.28 mmHg, respectively, and the others arterial blood parameters including pH, base excess, and bicarbonate were 7.54, 1.61, and 22.84 mmol $L^{-1}$, respectively. Also, the mean values of arterial blood electrolytes including $Na^+$, $K^+$, and $Cl^-$, were 143.25, 4.22, and 109.46 mmol $L^{-1}$, respectively, and the mean anion gap was 12.64 mmol $L^{-1}$. The present study determined the range of normal amounts of arterial blood gas, electrolytes and acid-base status in the healthy male sheep of Iranian Shall breed which could be used as a reference for further research on sheep diseases and to distinguish healthy status from disease.



## Introduction

Analysis of blood gas and electrolytes are useful for obtaining information about acid-base status, ventilation and gas exchange in the pulmonary circulation.[1,2] Blood gas and electrolytes allow quantitative and qualitative assessment of items such as metabolic acidosis, respiratory acidosis and mutual relationships between ventilation and oxygenation, and are a useful tool for clinical examination of the patient.[3] The metabolic and respiratory acidosis disorders are common in the veterinary patients, therefore, checking blood gas and electrolytes have become important diagnostic and monitoring tools.[4]

Measurements of arterial partial oxygen pressure ($PO_2$), arterial partial dioxide carbon pressure ($PCO_2$) and arterial oxygen saturation ($SaO_2$) provide valuable information on pulmonary function and tissue oxygenation. Technical advances and the modern devices of blood gas analysis enabled to measure a wide range of the blood variables including gas and electrolytes and determine the acid-base status.[5] Hence, out of the values of blood acidity (pH) and $PCO_2$, the variables such as plasma bicarbonate ($HCO_3^-$) concentration are obtained[2] which helps diagnose the metabolic disorders. Assessment of plasma bicarbonate concentration can be used to estimate the duration of primary respiratory disorders.

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Also, determining the amount of base excess (BE), which indicates the enhancement or decline of blood alkali reserves, is used to distinguish metabolic alkalosis from metabolic acidosis.[6]

In addition to the determination of factors related to blood gas, the quantification of sodium ($Na^+$), potassium ($K^+$) and chloride ($Cl^-$) concentrations and determination of the anion gap are useful as the auxiliary findings to diagnose several diseases in animals.

In sheep, the evaluation of the sick animal is usually done based on clinical examinations. However, assessment of blood gas and electrolytes provide a more accurate approach to determine the degree and nature of disorders.

Breed differences in sheep can affect the values of blood gas and electrolytes due to physiological, genetic and environmental reasons. Shall sheep be one of the native breeds of Iran, which has unique characteristics in the field of meat and wool production, the ability to adapt to the harsh weather, high fertility rate, and large size.[7]

Until now, the normal level of electrolytes and gas in the arterial blood has not been determined for Shall breed. Understanding the reference range of these items is valuable for effective management of the health and productivity of this breed.

The most appropriate reference range is the range obtained from a group of healthy animals that are in the same environmental and physiological conditions.[8,9] Therefore, the purpose of this research was determining level of arterial blood gas and electrolytes in the healthy male sheep of Iranian Shall breed to be able to obtain the normal range for this group breed of sheep.

## Materials and Methods

**Study design.** To do this study, 15 the healthy male sheep of Iranian Shall breed were selected in the mean aged of 4.76 ± 0.18 months and the mean weight of 32.46 ± 0.42 kg. All the sheep were kept in the same environment in terms of temperature and ventilation and with the same food ration. The daily ration composition for each sheep included 13.20% hay (350 g), 15.09% straw (400 g), 56.60% silage (1,500 g) and 15.09% concentrate (400 g). Also, the sheep had free access to water. Before starting the study, to ensure the health of the sheep, they were separately examined.

**Examination of sheep health.** The clinical conditions of various body systems were checked including cardio-vascular system (heart beat rate, strength of beat, rhythm, normal or adventitious sounds, color of mucous membranes, rate and strength of pulse and the state of vessels, especially the jugular vein and the capillary refill time, respiratory system (rate, normal or abnormal sounds, depth of breathing, cough and nasal discharge, observation nostrils and palpation of the larynx, cervical trachea and chest), digestive system (observation of the muzzle, mouth and teeth, the topographic position of rumen, reticulum, omasum, and abomasum, rate of rumen movements, the stool consistency, rectal temperature, and appetite), urogenital system (the position of urination, the color and volume of urine, palpation of kidney, bladder and testes), nervous system (consciousness level, standing and walking positions, head and neck position, function of cranial nerves, spinal reflexes, muscle tone and superficial and deep pain), visual system (observation of the eyes, eye discharge, visual reflexes, walking through the obstacles), auditory system (observation of the ears, ear discharge, auditory reflexes) and finally observation and palpation of the outer covering (skin and wool) and lymph nodes. Also, the hematological tests including complete blood count and air-dried blood smear were performed to check the blood factors, anemia and infection. Through the complete blood count test the amount of packed cell volume, hemoglobin, erythrocyte indices, red blood cell (RBC), total and differential white blood cell (WBC) were measured. The count of RBC, the morphological changes and erythrocyte indices were used to evaluate anemia, and total and differential WBC were used to evaluate bacterial and viral infections. Parasitic infections were checked by the test of air-dried blood smear. In addition, a fecal test was performed to check the parasite eggs in sheep and then the heart health of sheep was evaluated using electrocardiography and echocardiography. All animal maintenance and techniques were approved upon by the Animal Ethics Committee, Faculty of Veterinary Medicine, University of Tehran and done according to international protocol (Ethical code: IR.UT.VETMED.REC1402.031).

**Sampling and measurement of arterial blood gas and electrolytes.** After ensuring the health of each of the sheep, without the use of sedatives, the arterial blood sample was taken from the ear artery using an insulin syringe. In this way, first the sheep ears were shaved. Then, to prevent blood clotting in the insulin syringe, some heparin sulfate solution (as anticoagulant) was drawn into the syringe and its entire wall was smeared with heparin sulfate solution and ready to take a blood sample. The 0.50 - 1.00 mL of the arterial blood was collected and immediately delivered to the blood gas measuring device (OPTI CCA-TS, OPTI Medical Systems; IDEXX, Roswell, USA) and the parameters of pH, $PCO_2$, $PO_2$, $SaO_2$, BE, $HCO_3^-$ and the amount of electrolytes $Na^+$, $K^+$, $Cl^-$ and anion gap were measured and determined. The anion gap was calculated according to the following formula:

$$Anion\ Gap = (Na^+ + K^+) - (Cl^- + HCO_3^-)$$

**Statistical analysis.** The obtained data were statistically investigated by SPSS Software (version 24.0; IBM Corp., Armonk, USA). First, the normal distribution of the data was checked using the Kolmogorov-Smirnov test (a non-parametric statistical test). The correlation between the blood gas parameters and the weight of sheep

was evaluated through Pearson correlation coefficient. Descriptive statistics of the obtained results was expressed as mean ± SE, range (minimum and maximum) and confidence interval 95.00%.

## Results

**Confirmation of sheep health.** According to the clinical examinations and para-clinical evaluations, where there were abnormal signs or disorders in the sheep, it was excluded from the study and the other sheep was included. Therefore, the clinical examinations in all the sheep of this study showed that the checked symptoms were normal in all body systems, so that in cardiovascular system, heart beat rate and pulse rate were 81.86 ± 3.88 *per* min, the adventitious sounds were not heard, strength and rhythm of beat and pulse were normal, color of mucous membranes was pink, the jugular vein was without dilatation and pulse and capillary refill time was 1.33 ± 0.48 sec. In respiratory system, respiration rate was 22.13 ± 3.06 *per* min, abnormal sounds were not heard, and there were no cough and nasal discharge and inflammation or pain when observing and palpating the nostrils, larynx, cervical trachea and chest. In digestive system, the muzzle, mouth and teeth were healthy, the topographic position of rumen, reticulum, omasum, and abomasum were normal, rate of rumen movements were 2.66 ± 0.48 *per* 2 min, the stool consistency was in the form of small, firm, round pellets, rectal temperature was 38.13 ± 3.06 ˚C, and the appetite of all sheep was normal. In urogenital system, the position of urination and the color and volume of urine were normal, and there were no inflammation and pain in external palpation of kidneys, bladder and testes. In nervous system, the standing and walking positions and head and neck position were normal, the cranial nerves, spinal reflexes and muscle tone were healthy and there was no disorder, evaluating the tests of superficial and deep pain were associated with normal response and in general, all the sheep had a good level of consciousness. In the systems of visual and auditory, the different parts of the eyes and ears were healthy, the reflexes of visual and auditory were normal and there were no discharges from the eyes and ears. In observation and palpation of the outer covering (skin and wool), there was no wound, wool loss, or infection and the lymph nodes were normal in size.

Also, the laboratory assessments did not reveal any signs of anemia, infection and parasitic disease in the sheep. Therefore, the amount of packed cell volume, hemoglobin, erythrocyte indices, and RBC were within the normal range and there was no change in the shape of RBC, and anemia was not observed. In addition, the amount of total and differential WBC was within the normal range, and there were no bacterial or viral infections. In the fecal test, there were no parasite eggs in some sheep and in the others, the number of parasite eggs was very low, and in the test of air-dried blood smear, no blood parasite was observed and there were no the parasitic diseases in the sheep.

In the evaluation of the heart of sheep using electro-cardiography and echocardiography, no disturbance in heart rhythm, structure of heart cavities and valves was observed. The results of clinical examinations and the para-clinical evaluations confirmed the health of the used sheep in this study.

**The amount of arterial blood gas and electrolytes.** Statistical analysis using the Kolmogorov-Smirnov test showed that the distribution of arterial blood electrolytes and gas data was normal. Pearson's test did not display the correlation between the data of arterial blood electrolytes and gas with the weight of sheep.

The results of the arterial blood gas analysis of the healthy male sheep of Iranian Shall breed are presented in Table 1. The mean acidity (pH) of arterial blood was 7.54 (7.45 - 7.67), the mean $PCO_2$ of arterial blood was 28.26 (22.00 - 35.00) mmHg, the mean $PO_2$ of arterial blood was 61.58 (42.00 - 88.00) mmHg, the mean $SaO_2$ of arterial blood was 95.69% (85.00 - 99.00), the mean BE of arterial blood was 1.61 (0.70 - 3.80) mEq $L^{-1}$, and the mean $HCO_3^-$ of arterial blood was 22.84 (21.10 - 25.20) mmol $L^{-1}$.

The results of investigating the arterial blood electrolytes of the healthy male sheep of Iranian Shall breed are given in Table 2. The mean $Na^+$ of arterial blood was 143.25 (140 - 148) mmol $L^{-1}$, the mean $K^+$ of arterial blood was 4.22 (3.10 - 5.20) mmol $L^{-1}$, the mean $Cl^-$ of arterial blood was 109.46 (102 - 114) mmol $L^{-1}$, and the mean anion gap of arterial blood was 12.64 (9.40 - 17.80) mmol $L^{-1}$.

**Table 1.** The normal amount of arterial blood gases in the healthy male sheep of Iranian Shall breed as mean ± SE (confidence interval 95.00%: lower - upper limits) compared to the parameters of healthy sheep of other breeds.

| Breeds | pH | $PCO_2$ (mmHg) | $PO_2$ (mmHg) | $SaO_2$ (%) | BE (mEq $L^{-1}$) | $HCO_3^-$ (mmol $L^{-1}$) | References |
|---|---|---|---|---|---|---|---|
| **Iranian Shall** | 7.54 ± 0.01 (7.50- 7.57) | 28.26 ± 1.03 (26.20 - 30.26) | 61.58 ± 4.64 (53.08 - 71.16) | 95.69 ± 1.00 (93.46 - 97.38) | 1.61 ± 0.27 (1.12 - 2.21) | 22.84 ± 0.39 (22.08 - 23.64) | Present study |
| **Tuj** | 7.40 ± 0.05 | 41.85 ± 4.87 | 58.20 ± 10.27 | 82.34 ± 10.35 | 0.95 ± 4.43 | 25.94 ± 3.72 | 11 |
| **Suffolk** | 7.48 ± 0.007 | 32.10 ± 0.40 | 88.00 ± 0.83 | - | - | - | 12 |
| **Santa Inês** | 7.48 ± 0.01 | 33.71 ± 1.13 | 109.45 ± 5.08 | 97.72 ± 0.30 | 1.57 ± 0.49 | 23.94 ± 0.35 | 13 |
| **Merino** | 7.40 ± 0.05 | 42.10 ± 5.10 | 486.30 ± 51.00 | 99.90 (99.80- 99.90) | 1.40 ± 2.40 | - | 14 |
| **Bannur** | 7.39 ± 0.06 | 29.70 ± 1.33 | - | - | 1.80 ± 0.37 | 23.11 ± 0.52 | 15 |

$PCo_2$: Partial dioxide carbon pressure; $PO_2$: Partial oxygen pressure; SaO2: Oxygen saturation; BE: Base excess; $HCO_3^-$: Bicarbonate.

**Table 2.** The normal amount of arterial blood electrolytes in the healthy male sheep of Iranian Shall breed as mean ± SE (confidence interval 95.00%: lower – upper limits) compared to the parameters of healthy sheep of other breeds.

| Breeds | $Na^+$ (mmol $L^{-1}$) | $K^+$ (mmol $L^{-1}$) | $Cl^-$ (mmol $L^{-1}$) | Anion gap (mmol $L^{-1}$) | References |
|---|---|---|---|---|---|
| **Iranian Shall** | 143.25 ± 0.72 (141.75 - 144.66) | 4.22 ± 0.12 (3.99 - 4.46) | 109.46 ± 1.15 (107.23 - 111.69) | 12.64 ± 0.63 (11.56 - 14.04) | Present study |
| **Tuj** | 145.80 ± 1.80 | 4.80 ± 0.80 | - | - | 11 |
| **Merino** | 144.10 ± 1.50 | 3.00 ± 0.00 | 111.20 ± 3.70 | - | 14 |

## Discussion

The disorders of respiratory and acid-base are common in the veterinary and hospitalized patients,[4] and the arterial blood gas measurements are necessary to evaluate the cardiopulmonary function. From this point of view, the measurement of arterial blood gas is considered as the gold standard for determining the status of oxygenation, ventilation, gas exchange and acid-base homeostasis in the acute disorders as well as the critically ill patients, and the test repetition is a tool to monitor resuscitation efforts.[8]

The measurement of arterial blood acidity, $PO_2$, $PCO_2$ and $SaO_2$ provide the valuable information on respiratory status, pulmonary function and tissue oxygenation and the patient need for the oxygen therapy. The arterial $PO_2$indicates the blood oxygenation, the arterial partial carbon dioxide pressure indicates the adequacy of alveolar ventilation and pH indicates the condition of the blood in terms of acid and base.[10] In this study, the mean pH of arterial blood was determined 7.54, and it has been reported in the healthy sheep of Tuj breed 7.40,[11] in the healthy sheep of Suffolk breed 7.48,[12] in the healthy non-pregnant ewe of Santa Inês breed 7.48,[13] in the healthy 4 month-pregnant ewe of Merino breed 7.40,[14] and in the healthy sheep of Bannur breed 7.39.[15]

The $PO_2$ and $SaO_2$ of arterial blood are different. The $PO_2$ is the oxygen partial pressure in the arterial blood, which is assessed via the dissolved oxygen content in the blood (not the bound oxygen content to hemoglobin) and the blood temperature, and it can be considered as the driving force for the oxygen dispersion from the capillaries to the mitochondria.[16] The $SaO_2$ is stated as a percentage and demonstrates the total percentage of hemoglobin binding sites that are engrossed with oxygen. In general, the increasing $PaO_2$ is accompanied with the increasing arterial $SaO_2$.[17] Determination of $PO_2$ and $SaO_2$ are both the important measures of pulmonary function and oxygenation.

In this study, the mean $PO_2$ of arterial blood was 61.58 mmHg and the mean $SaO_2$ of arterial blood was 95.69%. The levels of arterial blood $PO_2$ and $SaO_2$ in the healthy Tuj breed were 58.20 mmHg (7.76 kPa) and 82.34%, respectively,[11] the level of arterial blood $PO_2$ in the healthy Suffolk breed was 88.00 mmHg,[12] the levels of $PO_2$ and $SaO_2$ in the healthy non-pregnant ewe of Santa Inês breed were 109.45 mmHg and 97.72%, respectively,[13] and in the healthy 4 months-pregnant ewe of Merino breed were 486.30 mmHg and 99.90%, respectively.[14]

Reportedly, in the female sheep of different breeds undergoing the laparotomy with inhalation anesthesia, the mean levels of $PO_2$ and $SaO_2$ were reported 93.00 mmHg and 93.00%, respectively, in the first hour of anesthesia.[18] In the present study, the level of $PO_2$ and $SaO_2$ indicated the normal function of lungs and the health of respiratory system in the male sheep of Iranian Shall breed.

The amount of BE indicates the enhancement or decline of the blood alkali reserves.[6] The normal amount of BE in large animals varies between 0 and 6 and is expressed in terms of mEq $L^{-1}$.[15] The BE more than 6 indicates the increasing base, metabolic alkalosis, and BE less than zero indicates the decreasing base, metabolic acidosis.

The mean amount of measured BE in this study was 1.61 mmol $L^{-1}$, which revealed the absence of metabolic alkalosis and acidosis in Iranian Shall breed. The mean amount of BE has been reported in the healthy Tuj breed, healthy non-pregnant ewe of Santa Inês breed, 4 month-pregnant ewe of Merino breed, and healthy Bannur sheep breed to be 0.95, 1.57, 1.40, and 1.80 mmol $L^{-1}$, respectively.[11,13-15] Grimm and Ganter showed that in the female sheep of different breeds undergoing the laparotomy with inhalation anesthesia, the amount of BE was reported to be 2.30 mmol $L^{-1}$ in the first hour of anesthesia.[18]

The actual amount of $HCO_3^-$ is calculated from pH and $PCO_2$ and the concentration of $HCO_3^-$ is considered a metabolic parameter whose changes display the presence of metabolic alkalosis or acidosis.[16] The mean $PCO_2$ and $HCO_3^-$ amounts of arterial blood in the present study were 28.26 mmHg and 22.84 mmol $L^{-1}$, respectively. The levels of arterial blood $PCO_2$ and $HCO_3^-$ in the healthy sheep of Tuj breed were 41.85 mmHg (5.58 kPa) and 25.94 mmol $L^{-1}$,[11] respectively, the arterial blood $PCO_2$ level in the healthy sheep of Suffolk breed was 32.10 mmHg[12] and in the healthy 4 months-pregnant ewe of Merino breed was 42.1 mmHg,[14] the levels of arterial blood $PCO_2$ and $HCO_3^-$ in the healthy non-pregnant ewe of Santa Inês breed were 33.71 and 23.94, respectively,[12] and in the healthy Bannur sheep breed were 29.70 and 23.11, respectively.[15] Thus, the reported $PCO_2$ and $HCO_3^-$ amounts in the arterial blood of male sheep of Iranian Shall breed showed the absence of metabolic alkalosis and acidosis and their health confirmed.

The blood gas analyses could also be used to diagnose diseases by measuring the amount of electrolytes including $Na^+$, $K^+$ and $Cl^-$ and determining the anion gap.[16] The mean amount of $Na^+$ (143.25 mmol $L^{-1}$), $K^+$ (4.22 mmol $L^{-1}$) and $Cl^-$ (109.46 mmol $L^{-1}$) reported in this study showed normal levels in the healthy male sheep of Iranian

Shall breed. The mean amount of arterial blood $Na^+$ and $K^+$ in the healthy sheep of Tuj breed were 145.80 and 4.80 mmol $L^{-1}$, respectively,[11] and the mean amount of arterial blood $Na^+$, $K^+$ and $Cl^-$ in the healthy 4 months-pregnant ewe of Merino breed were 144.1, 3.00 and 111.20 mmol $L^{-1}$, respectively.[14] The anion gap is a parameter to diagnose the cause of metabolic acidosis, which is the result of the difference between serum anions and cations.[16] In this study, the mean amount of anion gap was 12.64 mmol $L^{-1}$.

As mentioned, the normal range of arterial blood gas and electrolytes can be slightly different between sheep breeds and may be influenced by the various factors such as age, sex, pregnancy status and individual health of the sheep. In general, the determined amounts in this study could be applied to the healthy male sheep of Iranian Shall breed. However, it is necessary to consult a veterinarian for the specific guidance on the interpretation of blood gas and electrolytes amounts in the individual animals. In conclusion, this study determined the normal range of arterial blood gas and electrolytes in the healthy male sheep of Iranian Shall breed. Also, the present study showed that it was possible to measure the arterial blood gas and electrolytes easily and in a completely practical way with good quality and reproducibility in Iranian Shall breed and use to diagnose the lung diseases and the acid-base status. The results of this study could be used as a reference for the further research on the sheep diseases and distinguishing the healthy state from the disease.

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## Conflict of interest

Authors declare no conflict of interest.

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