

Clinical Study On the Impact of Amino Acids, Multi-Minerals, and Vitamins Combination Against Small Ruminants With Anemia In New Valley Governorate

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ABSTRACT: Anemia syndrome is defined as a reduction in erythrocyte mass, hematocrit, and hemoglobin (Hb) concentration and sometimes leads to tissue hypoxia. Domesticated sheep and goats are essential to food security and the national economy. The present study aimed to determine the clinical impact of anemia in sheep and goats from different localities in the New Valley governorate. 150 sheep and 360 goats were clinically examined. Blood and fecal samples were collected from multiple locations. Hematological variables, serum iron (Fe^{+2}), copper (Cu^{+2}), and vitamin B_{12} concentrations were estimated. Moreover, blood film and fecal examination were analyzed. Furthermore, the effect of amino acids, multi-minerals, and vitamins combination (AMVC) administration was studied on clinically anemic sheep and goats. The results revealed significant improvement in Hb, red blood cells count, mean corpuscular volume, and mean corpuscular Hb in anemic sheep and goats administrated AMVC compared to anemic ones. Moreover, serum Fe^{+2} , Cu^{+2} , and vitamin B_{12} levels were elevated. The current research found that anemic syndrome had significant detrimental effects on the health of sheep and goats in the New Valley governorate; however, using multivitamins, minerals, and essential amino acids in the ration can restore these effects.

KEYWORDS: Sheep; Goats; Anemia; Amino acids, Multi-minerals and vitamins combination.

1. Introduction

Small ruminant livestock breeding, including that of sheep and goats, has a significant economic activity, especially in Egypt [1]. They are regarded as a substantial supplier of food items like beef, milk, dairy products, and leather [2]. Anemia is a blood disorder and represents a common hazard in small ruminants [3]. Various factors cause anemia, such as blood loss, endoparasites, hemolysis, or decreased red blood cells (RBCs) production [4]. It is characterized by a low hemoglobin (Hb) concentration or hematocrit, which reduces RBCs ability to deliver oxygen [5]. Anemia is categorized differently depending on the pathophysiological feature or the complete blood count (CBC) parameters [6]. The first main classification, according to the pathophysiology, is subdivided into hemolytic type, where there is an elevation in RBCs break down, and hypo proliferative type, where RBCs creation is reduced [7].

Hemolytic anemia is occurred due to either inherited or acquired causes, such as autoimmune disease or infection cause [8]. However, hypo proliferative anemia is subdivided into aplastic anemia, nutritional deficiency anemia, and anemia connected to myelodysplastic syndrome [9]. The second classification of anemia based on the CBC aspect that subdivided it into subtypes according to mean corpuscular volume (MCV), mean corpuscular Hb (MCH) and mean corpuscular Hb concentration (MCHC) [10]. The MCV divides anemia into microcytic, normocytic, and macrocytic [11]. The MCH is categorized as anemia either to iron deficiency or underlying thalassemia [12]. However, based on the MCHC parameter, anemia is classified into hypochromic or spherocytosis types [13]. A balanced nutritional ration has a positive role in maintaining general animal performance and immune system improvement against infections [14]. Amino acids are organic molecules of protein-building units vital in cellular

function and immunity [15]. A deficiency of amino acids in ruminants leads to metabolic disorders, body weight loss, and reproduction diseases due to energy imbalance [16]. In the same way, minerals and vitamin supplementation to the ruminants contribute to preserving oxygen transport, deoxyribonucleic acid biosynthesis, and multiple neuronal functions [17]. In addition, they have a role in energy storage and utilization and regulation of the metabolic pathway of carbohydrates, protein, and lipids [18]. The current research focuses on the causative factors of anemia in sheep and goats in the New Valley governorate via their clinical examination for signs of anemia, and laboratory diagnosis of anemia at hematological and biochemical levels. In addition, this work investigated the effect of amino acids, multi-minerals, and vitamins consumption for anemic sheep and goats.

2. Materials and methods

2.1. Animal and study design

The study was done on 510 small ruminants, 150 sheep, and 360 goats, around 1-4 years old, and their body weight ranged between 20-60 kg. Sheep were chosen from El-Dakhla (n=54), El-Kharga (n=62), and Paris (n=34) cities in New Valley governorate. At the same time, goats were selected from El-Dakhla (n=97), El-Kharga (n=135), and Paris (n=128) cities in New Valley governorate. The experiment was conducted under the ethical guidelines of the Institutional Review Board, Faculty of Medicine, Assiut University, 04-2023-200293. The research was done between December 2020 and December 2021. Small ruminant case histories were initially documented, then a clinical examination was conducted. After that, the anemic animals were selected from each locality in the New Valley governorate based on the case history and clinical examination information. Also, control healthy animals were chosen from every city based on case history, clinical signs, and physical examinations (control group). After a complete survey of anemic sheep and goats, the animals were subdivided into two categories. Group 1, the anemic group, both sheep and goats suffered from anemia,

and Group 2, the treated group, where sheep and goats in this group were treated daily for one month with amino acids, multi-minerals, and vitamins combination (AMVC), which obtained from Animal Production Research Institute, Regional Center for Food and Feed, Agriculture Research Center, Egypt, according to the manufacture of instruction, where 1 kg of AMVC was added to 1 ton of ration. This combination consisted of 30000 mg methionine, 5000 mg lysine, 120000 mg manganese, 100000 mg zinc, 80000 mg iron, 13000 mg copper, 1500 mg iodine, 300 mg cobalt, 350 mg selenium, 1100000 I/U vitamin A, 2200000 I/U vitamin D₃, and 100 mg vitamin K₃. The animals in group 1 were further subdivided according to Hb concentration into two groups, anemic Hb concentration below 8 group and anemic Hb concentration from 8 to 10 group.

2.2. Physical examination and blood and fecal sampling

Clinical examinations of small ruminants were performed by investigating visible mucous membranes, extremities, physical condition, general appearance, gait, posture, anus, and skin and eyes lesions. Blood samples were collected from the jugular vein before and after animal treatment, and all attempts were made to keep animals under minimum stress. Two types of test tubes were used to collect blood samples, one was with ethylene diamine tetra acetic acid (EDTA) that serves as an anticoagulant, and the second was without anticoagulant and maintained in an incline for 20 minutes at room temperature. Tubes with anticoagulant were stored without centrifugation and utilized for complete blood picture count (CBC) and blood film; however, the second tubes were centrifuged, and the clear serum was harvested and stored at -20°C until analysis. The selected samples for biochemical investigation depended on CBC results. Blood and serum samples were also taken from control healthy sheep and goats. Moreover, 5 gm of fresh fecal samples were obtained from suspected diseased animals before and after treatment to detect internal parasites.

2.3. Hematological examination

The hematological variables were estimated in the Research Laboratory, Faculty of Veterinary Medicine, New Valley University, with an Auto hematology Analyzer (KT-60, China). These parameters comprised red blood cells count (RBCs), Hb, MCV, and MCH concentration.

2.4. Parasitological examination of blood smears

Thin blood smears were made using blood samples in EDTA tubes. Then, the smear was air dried and fixed in absolute ethyl alcohol (Sigma Aldrich Co. (USA)) for 5 mins., and then it stained with 10% Giemsa (Sigma Aldrich Co. (USA)) for 20 mins. The slides were inspected with an oil immersion lens at a magnification of $\times 1000$ to determine the parasite in erythrocytes [19]

2.5. Serum biochemical analysis

All biochemical parameters were estimated using Biochemistry Analyzer (JENWAY 61431, 6705, UK) in the Research Laboratory, Faculty of Veterinary Medicine, New Valley University. Serum Fe^{2+} and Cu^{2+} concentrations were assessed photometrically as kits (Spectrum Co. (Germany)) manufacturers instructed. Furthermore, serum vitamin B_{12} was also determined by the ELISA technique, where the kits were obtained from Biovision Co. (USA). The assay complied with the manufacturer's instructions and employed the quantitative sandwich enzyme immunoassay procedure. Antibody specific for vitamin B_{12} has been pre-coated onto separate microplates. The test is based on combining samples or standards with vitamin B_{12} antibodies. The density of color is proportional to the amount of vitamin B_{12} captured from the samples. The concentration of serum vitamin B_{12} in ng/ml was determined using the standard curve.

2.6. Parasitological examination of fecal samples

Fecal samples of studied animals were qualitatively examined by wet preparation direct smear and sedimentation technique. In brief, in the direct smear method, 5 gm of feces were mixed with water on the slide that was examined via a low-power microscope ($\times 10$) to detect the presence of either worm eggs, larvae and/or protozoa trophozoites

and cysts [20]. However, 5 gm of feces was weighed and mixed with the saturated salt solution in the sedimentation technique. The mixture was passed through a gauze, the supernatant was discarded, and the sediment dissolved in H_2O . After that, the supernatant and sediment were again stained by trichrome stain (Sigma Aldrich Co. (USA)) and examined under a microscope to detect eggs of parasitic helminths [21]

2.7. Statistical analysis

The acquired data underwent statistical analysis using SPSS (Version 25), One way ANOVA. The results were displayed as Mean \pm SE, and the significant P value was < 0.05 .

3. Results

3.1. Clinical findings

The data obtained from case history exposed that sheep suffered from symptoms of anemia in percentages 20.37, 25.81, and 17.35 in El-Dakhla, El-Kharga, and Paris, respectively, from a total number of examined sheep (54, 62, and 34, in El-Dakhla, El-Kharga, and Paris, respectively). Further, it was observed the percentage of anemia signs in goats was 7.22, 22.22, and 23.44 in El-Dakhla, El-Kharga, and Paris, respectively, from the whole number of inspected goats (97, 135, and 128, in El-Dakhla, El-Kharga, and Paris, respectively). Further, 5.33% of clinically examined sheep suffered from pale mucous membrane and diarrhea, 4% were emaciated and suffered from alopecia symptoms, 2.67% showed ticks presence, and 0.67% were affected with mites. At the same time, in goats, pale mucous membrane, emaciation, ticks, and mites' infestation was noted in 1.94, 5.83, 3.06 and 3.33%, respectively, of the total number of inspected goats. However, only 2.22% of goats suffered from diarrhea and alopecia symptoms (Table 1). Based on all clinical examinations, the present study found 22.0% of sheep and 18.61% of goats from the investigated small ruminants in El-Dakhla, El-Kharga, and Paris regions, displayed clinical anemia symptoms (Fig. 1).

3.2. Laboratory findings

The current data exhibited a considerable decrease in Hb level in sheep nearly (42.63-32.59%) in anemic samples compared to control sheep. The group of sheep that exhibited a decrease of Hb concentration by 42.63% showed a decline in RBCs count, MCV, and MCH by 28.13, 40.14, and 28.30%, respectively. However, sheep had a decrease in Hb concentration by 32.59%, and the reduction was by 41.67, 55.60 and 34.29% in RBCs count, MCV, and MCH, respectively. Moreover, after treating anemic sheep with AMVC, there was an increase in Hb, RBCs count, MCV, and MCH by 33.19, 21.20, 86.69 and 43.90%, respectively, in the group of sheep that revealed a decline in Hb value by 42.63%. Moreover, there was an elevation of RBCs count, MCV, and MCH by 7.57, 1.96, and 6.56-folds, respectively; however, there was no significant difference in Hb concentration (Table 2). Additionally, the results showed a significant decline in Hb concentration in goats ranging from 40.41% to 28.76% compared to control goats. It was noted significant drop in RBCs count, MCV, and MCH by 37.43, 62.38 and 68.23%, respectively, in goats suffered from a reduction in Hb concentration. Treated goats suffered from a decrease in Hb concentration by 40.41%, and 28.76% displayed an increase in Hb, RBCs count, MCV, and MCH, and the data was demonstrated in (Table 3). Moreover, the results revealed the presence of 18% of examined sheep and 13% of examined goats had blood parasites before AMVC administration (Table 4). After AMVC, those percentages were reduced to 2% and 3% in sheep and goats, respectively. Serum data of Fe²⁺, Cu²⁺, and vitamin B₁₂ analysis of sheep in Table 5 demonstrated that a reduction of serum Fe²⁺, Cu²⁺, and vitamin B₁₂ by 20.23, 22.67 and 92.42% in the anemic sheep group had a discount of Hb concentration by 42.63%, respectively, in comparing to control one. Also, the anemic sheep group had a decrease of Hb concentration by 32.59%; there was a decrease in serum Fe²⁺, Cu²⁺, and vitamin B₁₂ by 55.92, 66.34, and 78.87%, respectively, compared to control goats. The concentration of Fe²⁺, Cu²⁺, and vitamin B₁₂ was determined after treatment by AMVC. It was

noted that Fe²⁺ and vitamin B₁₂ concentrations were increased by 0.55 and 4.42-folds in the anemic sheep group had a reduction of Hb concentration by 42.63%; however, there wasn't a significant variation in Cu²⁺ concentration when compared with non-treated goats. In the same way, treated sheep had a drop of Hb concentration by 32.59%, revealing an increase of serum Fe²⁺, Cu²⁺, and vitamin B₁₂ levels by 0.93, 2.24, and 8.82-folds compared with other sheep before treatment. Serum Fe²⁺, Cu²⁺, and vitamin B₁₂ assays in goats exhibited a decline of 60.33, 42.53 and 84.0%, respectively in goats had a 40.41% decrease in Hb value and, they reduced by 43.50, 24.84 and 77.50% in goats had 28.76% diminution of Hb concentration, in comparison to control goats. Using AMVC leads to a 16.58, 0.69, and 0.98- folds increase of Fe²⁺, Cu²⁺, and vitamin B₁₂ concentrations in goats, showing a 40.41% decline in Hb. Additionally, the serum Fe²⁺, Cu²⁺, and vitamin B₁₂ concentrations rose by 2.40, 0.84, and 3.12-folds in goats and had a 28.76% decrease in Hb value compared to those before treatment (Table 6). Further, fecal examination by direct smear and sedimentation techniques revealed the absence of oocytes, larvae, or worms in both anemic sheep and goat samples.

Table 1: Clinical findings of studied sheep and goats

Factor		Sheep	Goat
Total number tested (No)		150	360
Clinical disease (No)		33	67
Clinical symptoms (No)	Pale mucous membrane	8	7
	Diarrhea	8	8
	Emaciation	6	21
	Alopecia	6	8
Ticks infestations (No)		4	11
Mite infestation (No)		1	12

4. Discussion

Sheep and goats have economic importance as they are characterized by their small body size, allowing easy investment into different farming systems. They are also

Table 2: Mean values (± SE) of hematological parameters in control healthy and anemic sheep before and after treatment

Hematological parameters	Control (n= 12)	Anemic Hb concentration below 8 (n= 24)		Anemic Hb concentration from 8 to 10 (n= 24)	
		Before treatment (n= 12)	After treatment (n= 12)	Before treatment (n= 12)	After treatment (n= 12)
Hb (g/dl)	12.55 ± 0.88	7.20 ± 0.25 ^a	9.59 ± 0.20 ^{a*}	8.69 ± 0.10 ^a	8.46 ± 0.07 ^a
RBCs (10 ⁶ /ul)	7.68 ± 0.55	5.52 ± 0.23 ^a	6.69 ± 0.54 ^{***}	4.48 ± 0.20 ^a	7.87 ± 0.13 [*]
MCV (fL)	43.67 ± 0.24	26.14 ± 0.23 ^a	48.80 ± 0.19 [*]	19.39 ± 0.18 ^a	57.30 ± 0.12 ^{c*}
MCH (pg)	16.36 ± 0.28	11.73 ± 0.13 ^a	16.88 ± 0.08 [*]	10.75 ± 0.29 ^a	17.80 ± 0.11 [*]

Values represent mean ± SE. Means with different superscript ^ap<0.001, ^bp<0.01, ^cp < 0.05 are significantly different compared to control. Means with different superscripts *p<0.001, **p<0.01, ***p < 0.05 are significantly different compared to the anemic sheep after treatment to the anemic sheep before treatment.

Table 3: Mean values (± SE) of hematological parameters in healthy control and anemic goats before and after treatment

Hematological parameters	Control (n= 12)	Anemic Hb concentration below 8 (n= 24)		Anemic Hb concentration from 8 to 10 (n= 24)	
		Before treatment (n= 12)	After treatment (n= 12)	Before treatment (n= 12)	After treatment (n= 12)
Hb (g/dl)	11.58 ± 0.77	6.90 ± 0.29 ^a	9.25 ± 0.03 ^{a*}	8.25 ± 0.03 ^a	8.55 ± 0.26 ^a
RBCs (10 ⁶ /ul)	8.79 ± 0.32	5.50 ± 0.35 ^a	9.27 ± 0.25 ^{***}	5.90 ± 0.46 ^a	12.81 ± 0.39 ^{c*}
MCV (fL)	41.68 ± 0.66	15.68 ± 0.32 ^a	44.07 ± 0.53 [*]	12.75 ± 0.43 ^a	47.40 ± 0.29 [*]
MCH (pg)	14.89 ± 0.30	4.73 ± 0.96 ^a	14.09 ± 0.57 [*]	6.40 ± 0.17 ^a	14.66 ± 0.71 [*]

Values represent mean ± SE. Means with different superscript ^ap<0.001, ^bp<0.01, ^cp < 0.05 are significantly different compared to control. Means with different superscripts *p<0.001, **p<0.01, ***p < 0.05 significantly differ from the anemic goats after treatment to anemic goats before treatment.

Table 4: Prevalence of blood parasites in clinically diseased sheep and goats by blood film examination before AMVC administration

Animals	Examined animals			Parasite species							
	Total	+ve	%	Babesia Spp.		Theilaria Spp.		Anaplasma Spp.		Mixed infection	
				+ve	%	+ve	%	+ve	%	+ve	%
Sheep	33	6	18	1	3	2	6	2	6	1	3
Goats	67	9	13	2	3	3	4.48	3	4.48	1	1.49

Table 5: Mean values (± SE) of serum Fe²⁺, Cu²⁺, and vitamin B₁₂ concentrations in healthy control and anemic sheep before and after treatment

Studied parameters	Control (n= 12)	Anemic Hb concentration below 8 (n= 24)		Anemic Hb concentration from 8 to 10 (n= 24)	
		Before treatment (n= 12)	After treatment (n= 12)	Before treatment (n= 12)	After treatment (n= 12)
Fe ²⁺ (µg/dl)	100.24 ± 0.66	79.96 ± 1.74 ^b	84.29 ± 1.88 ^c	44.19 ± 1.30 ^a	85.14 ± 1.19 ^{c*}
Cu ²⁺ (µg/dl)	70.04 ± 1.09	54.16 ± 0.97	52.22 ± 1.31	23.57 ± 0.21 ^a	76.36 ± 1.43 [*]
vitamin B ₁₂ (ng/ml)	1351.25 ± 0.89	102.39 ± 0.79 ^a	555.50 ± 0.58 ^{a**}	285.55 ± 1.88 ^a	537.40 ± 0.98 ^{a**}

Values represent mean ± SE. Means with different superscript ^ap<0.001, ^bp<0.01, ^cp < 0.05 are significantly different compared to control. Means with different superscripts *p<0.001, **p<0.01, ***p < 0.05 are significantly different compared to the anemic sheep after treatment to the anemic sheep before treatment.

suitable for wool, meat, and milk sources [22]. The clinical examination for signs of anemia was performed on sheep and goats, and the results revealed the presence of

Table 6: Mean values (± SE) of serum Fe²⁺, Cu²⁺, and vitamin B₁₂ concentrations in healthy control and anemic goats before and after treatment

Studied parameters	Control (n= 12)	Anemic Hb concentration below 8 (n= 24)		Anemic Hb concentration from 8 to 10 (n= 24)	
		Before treatment (n= 12)	After treatment (n= 12)	Before treatment (n= 12)	After treatment (n= 12)
Fe ²⁺ (µg/dl)	65.81 ± 0.41	26.11 ± 0.56 ^a	69.41 ± 0.91 ^{a**}	37.18 ± 0.89 ^a	126.63 ± 0.88 ^{a*}
Cu ²⁺ (µg/dl)	57.04 ± 0.90	32.78 ± 0.77 ^a	55.29 ± 0.70 ^a	42.87 ± 0.23 ^a	78.89 ± 0.92 ^{a*}
vitamin B ₁₂ (ng/ml)	734.50 ± 0.44	117.50 ± 0.18 ^a	232.45 ± 0.46 ^{a*}	165.25 ± 0.85 ^a	681.0 ± 0.68 ^{b*}

Values represent mean ± SE. Means with different superscript ^ap<0.001, ^bp<0.01, ^cp < 0.05 are significantly different compared to control. Means with different superscripts *p<0.001, **p<0.01, ***p < 0.05 significantly differ from the anemic goats after treatment to the anemic goats before treatment.

pale mucous membranes, emaciation, diarrhea, alopecia, ticks, and mites. Our observed clinical signs agreed with [23], who recorded that anemic sheep and goats exhibited emaciation, including pale mucus, loss of appetite, diarrhea, and easily separated wool. The incidence of anemia is ascribed to reduced activity of the cytochrome oxidase enzyme, leading to defects in phospholipids biosynthesis and hence the abnormal shape of RBCs and delay its maturation [24]. Our hematological analysis revealed the



Figure 1: Clinical signs of anemia in sheep and goats

following, decline of Hb value and RBCs count in anemic studied animals, and this may attribute to the reduction of Cu^{2+} concentration that is responsible for normal Fe^{2+} absorption and impairing the ferroxidase enzyme activity that is required for Hb biosynthesis [25]. Defects of Fe^{2+} absorption and metabolism cause irregular pathways of Hb synthesis [26]. Further, it was noted that Cu^{2+} deficiency causes elevation of RBCs hemolysis and erythrocytes peroxidation, promoting anemia incidence [27]. Our current data align with the prior study of [28]. Moreover, the existing data exhibited a reduction of vitamin B_{12} concentration in anemic sheep and goats, and this considers another cause of anemia due to the abnormal erythropoiesis process [29]. Administration of AMVC to anemic sheep

and goats causes enhancement of anemic conditions via increased RBCs count and concentration of Hb, MCV, MCH, Fe^{2+} , Cu^{2+} , and vitamin B_{12} . The positive influence of AMVC is chiefly related to its active ingredients. AMVC contains methionine, an essential amino acid that plays a vital role in hematopoiesis [30]. Another component of AMVC is Fe^{2+} which helps develop and mature erythrocytes and normal Hb biosynthesis [31]. Also, the presence of Cu^{2+} in the AMVC assisted in improving the studied parameters of anemia, which was agreed with [32]), who observed a significant increase of Hb after Cu^{2+} administration. Cu^{2+} is a component of cytochrome oxidase enzyme and superoxide dismutase that is required for Hb synthesis [33]. Further presence of cobalt (Co^{2+}) in

AMVC was vital in normal blood cell formation as Co^{2+} is a component of vitamin B_{12} [34]. Selenium (Se^{2+}) is an essential glutathione peroxidase enzyme component that protects Hb and red cell membranes from oxidative damage [35]. Further, both Se^{2+} and vitamin A are potent antioxidants that hinder peroxide formation, so they preserve the RBCs against hemolysis [36]. Vitamin A is essential for transferrin synthesis required for Fe^{2+} transport [37]. Zinc in the AMVC also helped in the mobilization of vitamin A in the liver [38].

5. Conclusion

In the New Valley governorate, micronutrients are essential for ruminants' nutrition, especially for alleviating malnutrition, the leading cause of anemic syndromes among small animals. The current study showed that a high incidence of anemia is related to copper and vitamin B_{12} deficiency. Administration of amino acids, multi-minerals, and vitamins combination improves the hematological and biochemical parameters. Therefore, regulatory examination and health status monitoring are advised to increase these animals' economic profit and production.

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7. Conflict of interest disclosure

The authors declare no conflicts of interest associated with this manuscript.

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