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Effect Of Dietary Ascorbic Acid (Vitamin C) On Performance And Immunity Of Heat Stressed Broiler Chickens

To Department of Veterinary in (Shaqlawa Technical College) in partial Fulfilment of the Requirement of Bachelor's Degree in Veterinary

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

يَحْسَــبُونَ الْأَحْزَابَ لَمْ يَـذْهَبُوا وَإِنْ يَأْتِ الْأَحْزَابُ يَوَدُّوا لَوْ أَنَّهُمْ بَادُونَ فِي الْأَعْرَابِ يَسْأَلُونَ عَنْ أَنْبَائِكُمْ وَلَوْ كَانُوا فِيكُمْ مَا قَاتَلُوا إِلَّا قَلِيلًا.

صَبَلَ قَبَاللَّهُ اللَّهُ العُظَمِينِ،

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Research entitled (Effect of Dietary Ascorbic Acid (Vitamin C) On Performance and Immunity of Heat Stressed Broiler Chickens) under my Supervision in Shaqlawa Technical College Veterinary Department Submitted for the Purpose of obtaining a bachelors in (Veterinary).

Signature

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Date: / /2024

Certification

I certify that this study was prepared by students (Hangaw Muhammad Mustafa & Dler Hamza Hamad), under my supervision at the Shaqlawa Technical College Veterinary Department, in partial Fulfillment of the requirements for the degree of Bachelor in Veterinary.

Signature

Dr. Nawal Kamal Shokry Barzani

Dedication

Dedicate this search.....

- Our dear teacher, Mr. (Dr. Nawal Kamal Shukri),
- My father and my mother, who do not stop giving me affection, hope and support,
- ➤ For all my family, the symbol of love and tenderness,
- > My dear teachers who guided me to the right path,
- My friends who encouraged me and supported me throughout the research.

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List Of Contents

Contents
List of tables
Abstract
Chapter One1
Introduction1
Chapter Two2
2.1 Poultry Industry
2.2 Poultry and Immune System2
2.3 Sources of Vitamin C
2.3.1 A-Food
2.3.2 B- Dietary supplements
2.4 Role of Vitamin C on broiler performance
2.5 What is the role of Vitamin C as antioxidant
2.6 The Mechanism of Ascorbic Acid4
2.7 Avian immune system
2.8 Measures of immunity5
2.9 Effect of Vitamin C on immune response
2.10 Effect of dietary vitamin C on Immune response of heat stressed broiler7
CHAPTER THREE
RESULTS 8
3.1. Feed intake
3.2 Body weight gain
3.3 Feed conversion Ratio
Chapter Four11
4.1 CONCLUSION
4.2 RECOMMONDATION11
References

List of tables

Table 1: Effect of different levels of ascorbic acid on weekly feed intake 10
Table 2: Effect of different levels of ascorbic acid on weekly Weight gain
Table 3: Effect of different levels of ascorbic acid on weekly feed conversion ratio of broiler
chicks (g feed/g body weight) during summer11
Table 4: Effect of different levels of ascorbic acid on lymphoid organs of broilers (g/bird)11

Abstract

Ascorbic acid, widely known as vitamin C, is an essential nutrient for animals such as poultry. Ascorbic acid in poultry feed improves animal health and thus increases the growth performance of birds. Ascorbic acid can be used in the form of synthetic products or can be naturally obtained from fruits and plants. It is soluble in water and can be easily administered in drinking water and the diet. Poultry can synthesize ascorbic acid in the body. However, the performance of the animals can be improved by adding ascorbic acid to their diet. In addition, ascorbic acid is called an antioxidant and an anti-inflammatory. This increases their resistance to disease during the transition season. Ascorbic acid supplementation positively affects the stress response, especially during the dry season in tropical countries. Furthermore, supplementing ascorbic acid in the poultry's diet improves resistance to diseases, regulates stress, and helps in the body's oxidation process. Ultimately, this enhances the laying rate, egg hatch performance, and higher poultry productivity. For layers at the end of the laying period, it helps increase the quality of the eggshell and reduces the proportion of broken eggs. Ascorbic acid has a strong relationship with other vitamins such as vitamin E and other substances such as zinc, safflower oil, folic acid, and a fibrous diet. This review aims to synthesize all the information of ascorbic acid in the poultry's diet, thereby providing the general role of ascorbic acid for the poultry industry.

Chapter One

Introduction

The poultry industry has advanced over the years. Although poultry's productivity has improved, it is still relatively low. In addition, the epidemic situation in poultry production is complex, and the diseases are not fully controlled. Therefore, the cost of veterinary drugs is high, which reduces the efficiency of poultry production.

Ascorbic acid is called an antioxidant compound with a chemical formulation and the properties. It has antioxidant and anti-inflammatory effects. Therefore, it is effective for poultry in cases of inflammation, oxidative stress, and infection [1]. Ascorbic acid is not an essential nutrient for poultry as it can be synthesized from poultry through the biosynthetic pathway [2]. However, it is considered an essential nutrient in two cases: (1) It is important when birds do not synthesize enough ascorbic acid in the native synthesis process and (2) the requirement of poultry for ascorbic acid is high in case of hot weather or stressful condition [3]. The role of ascorbic acid has been demonstrated in previous studies. First, ascorbic acid is added as an additional nutrient to the diet to improve poultry performance by improving body weight and reducing mortality [3]. Furthermore, the use of ascorbic acid was aimed at improving the immune response and antioxidant capacity of birds [4-7]. In particular, in a heat stress environment, ascorbic acid had contributed to the energy supply of poultry birds by corticosterone biosynthesis [4]. In addition, the use of ascorbic acid also plays a crucial role in the treatment and prevention of Salmonella enteritidis [8]. Modulating physiological functions have also been recorded in some previous studies [5,6]. According to Gan et al. [9], ascorbic acid supplementation increased spleen ascorbic acid level and serum immunoglobulin G (IgG) levels.

The use of ascorbic acid to support poultry animals in adverse conditions, especially under heat stress conditions, is essential; it is one of the important processes during the rearing period. Furthermore, the use of ascorbic acid in the poultry's diet helps improve poultry's performance and health. This review aims to synthesize all the information of ascorbic acid in the poultry's diet, thereby providing the general role of ascorbic acid for the poultry industry.

Chapter Two

2.1 Poultry Industry

Poultry industry is a broad specialization, there are many sub-sectors in the poultry industry in which one could go fully into. When poultry farming is mentioned, most people do misconstrue the meaning, some might just think its chicken rearing or turkey rearing or even geese rearing alone, but poultry farming is a very wide agro business with different sectors [24].

Disease control, high production, product quality, and reasonable production costs have been the recent main goals of the poultry industry. Hence, meeting per capita consumption and welfare to humans necessitates continuous efficient and goal-oriented healthcare to control disease spread and decrease the application of antibiotics [22].

These endeavours will include the launch of programs to control infectious diseases, face the constant changes in political and social conditions, address consumers' perceptions about animal welfare, and ensure the safety and security of foods and environmental defense issues. In addition, the continuous increase in the costs of feedstuffs (and thus feeds and foods) remain prominent issues [23].

2.2 Poultry and Immune System

The immune system in chickens can be divided into primary immune organs and lymphoid tissue. The primary immune structures are the thymus, where T lymphocytes are produced and mature; the bursa of Fabricius, where B lymphocytes mature; and the bone marrow, where blood cell precursors are produced. In addition, during the embryonic development of chickens, the source of maternal antibodies is the yolk sec. Primary lymphoid organs mainly act as a center for the production and maturation of adaptive immune cells. Secondary lymphoid tissues specialize in controlling immune responses. They activate immune effector cells, such as lymphocytes [25]. After maturing in primary lymphoid organs, T and B lymphocytes re-enter the bloodstream and colonize secondary lymphoid tissues to facilitate antigen presentation to lymphoid cells and initiate and regulate the adaptive immune response.

The fundamental difference between the immune system in mammals and chickens is the lack of encapsulated lymph nodes. Instead, we find in them "diffuse" lymphoid tissue and its clusters in organizations such as Peyer patches, cecal tonsils, and Meckel's diverticulum [26]. Lymphoid tissues include the spleen and mucosa-associated lymphoid tissues (MALT), also classified as the mucosal immune system (MIS). Lymphoid tissues in mucous membranes lining

systems associated with nutrition (gut-associated lymphoid tissue—GALT), respiration (nasal-associated lymphoid tissue—NALT, bronchus-associated lymphoid tissue—BALT), and vision (conjunctiva-associated lymphoid tissue—CALT). In chickens, these tissues are immunologically well-developed and are the first line of defense against pathogens (27).

2.3 Sources of Vitamin C

2.3.1 A-Food

Fruits and vegetables are the best sources of vitamin C. Citrus fruits, tomatoes and tomato juice, and potatoes are major contributors of vitamin C to the American diet. Other good food sources include red and green peppers, kiwifruit, broccoli, strawberries, Brussels sprouts, and cantaloupe. Although vitamin C is not naturally present in grains, it is added to some fortified breakfast cereals. The vitamin C content of food may be reduced by prolonged storage and by cooking because ascorbic acid is water soluble and is destroyed by heat. Steaming or microwaving may lessen cooking losses. Fortunately, many of the best food sources of vitamin C, such as fruits and vegetables, are usually consumed raw. Consuming five varied servings of fruits and vegetables a day can provide more than 200 mg of vitamin C.

2.3.2 B- Dietary supplements

Supplements typically contain vitamin C in the form of ascorbic acid, which has equivalent bioavailability to that of naturally occurring ascorbic acid in foods, such as orange juice and broccoli. Other forms of vitamin C supplements include sodium ascorbate; calcium ascorbate; other mineral ascorbates; ascorbic acid with bioflavonoids; and combination products, such as Ester-C, which contains calcium ascorbate, dehydroascorbate, calcium threonate, xylonate and lyxonate. A few studies in humans have examined whether bioavailability differs among the various forms of vitamin C. In one study, Ester-C and ascorbic acid produced the same vitamin C plasma concentrations, but Ester-C produced significantly higher vitamin C concentrations in leukocytes 24 hours after ingestion. Another study found no differences in plasma vitamin C levels or urinary excretion of vitamin C among three different vitamin C sources: ascorbic acid, Ester-C, and ascorbic acid with bioflavonoids. These findings, coupled with the relatively low cost of ascorbic acid, led the authors to conclude that simple ascorbic acid is the preferred source of supplemental vitamin C.

2.4 Role of Vitamin C on broiler performance

Quarles and Adrian (1989) indicated that when vitamin C was supplemented at a level of 976 ppm/128 gal in the drinking water for 24 hrs prior to pickup for slaughter carcass yield was significantly increased.500 mg/kg of ascorbic acid supplementation resulted in an improved weight gain until the fifth week, and body weight at slaughter, carcass weights and carcass yield were not affected by ascorbic acid supplementation as reported [43].

Raja and Qureshi (2000) reported a significant improvement in body weight of birds provided with ascorbic acid in the feed [42]. suggested that improvement in body weight may also related to vitamin C protection of cell membrane integrity. [41] postulated that the suppression of adrenocortical steroidognesis by vitamin C may constitute the primary reason that this vitamin can ameliorate the negative effects of stress.

2.5 What is the role of Vitamin C as antioxidant

Vitamin C is an antioxidant that helps protect your cells against the effects of free radicals' molecules produced when your body breaks down food or is exposed to tobacco smoke and radiation from the sun, X-rays or other sources. Free radicals might play a role in heart disease, cancer and other diseases. Vitamin C also helps your body absorb and store iron.

2.6 The Mechanism of Ascorbic Acid

Ascorbic acid is considered a powerful water-soluble antioxidant that neutralizes reactive oxygen species (ROS) and reduces oxidative stress in various studies [27,28]. Moreover, when it comes to biological systems, ascorbic acid is a powerful reducing agent and a free radical scavenger [29]. It serves as the body's first line of defense against free radicals, and it also protects proteins and lipid membranes from oxidative stress. As a water-soluble compound, ascorbic acid plays a role in body cells' interior and exterior parts, where it neutralizes free radicals and prevents free radical damage. In addition, ascorbic acid is an excellent source of electrons for free radicals, which are always looking for an electron to restore its stability. Ascorbic acid can donate electrons to free radicals, thereby reducing their reactivity [27]. Moreover, the activity of ascorbic acid is a result of the acid's ability to act as an electron transfer agent (as a reducing agent) in the biological system [30]. This ability is considered a key enzyme cofactor,

which normally helps to increase the enzyme activity by keeping the reduced forms of iron (Fe2+) and copper (Cu+) at the active site of the enzyme [31].

Although this cofactor has been recorded for many enzymatic functions, it is perhaps best known for its role in collagen protein production [31]. In addition to increasing iron absorption under normal conditions, ascorbic acid increases iron absorption from non-heme iron sources by reducing Fe3+ to Fe2+ [32]. When redox-active ions are present in the environment, ascorbic acid functions as a pro-oxidant contributing to the formation of hydroxyl radicals, which can result in lipid, DNA, or protein oxidation, among other things [27,28]. Due to its reducing capacity, ascorbic acid is an important biological antioxidant that protects against ROS that gains electrons from adjacent biological systems and other harmful substances [32]. In the events where ascorbic acid or other antioxidants are absent, ROS causes unwanted alterations to structures in biology such as DNA, RNA, proteins, and lipids. These modifications can cause mutations [30,32,33].

2.7 Avian immune system

The immune system of the fowl is dependent on specialized microenvironments. It formed of the major lymphoid tissues, the thymus and the bursa of fabricus and secondary lymphoid tissues, Spleen, Lymph Node, and Cecal Tonsil [17].

The thymus has been demonstrated functionally by specific stimulation of Tlymphocyte. It consisted of seven lobes developed along each side of the jugular veins. The thymus possessed cortical and medullary regions an isolated protein, the putative avian thymic hormone, located in the thymus and blood, stimulated bone marrow cells to express T-cell markers [18].

The initial descriptive study of the bursa was by Hieronymus Fabricius the bursa is a dorsal diverticulum of the proctodaeal region of the cloaca [19]. The main function of the bursa is the immunological education of prebursal stem cells to form immunologlobulin.

The avian spleen like the mammalian spleen possesses red and white pulp. Adjacent to the dorsal surface of the right lobe of the liver and dorsal to the proventriculus is the reddish-brown oval spleen [20]. Secondary lymphoid tissues where they produce immunoglobulins a specific antibody and perform other immunological function [21].

Blood heterophils are granulated leukocytes formed from myelocytes in the bone marrow. They are phagocytic cells designed to define the organism against infections by bacteria, viruses, or foreign particles. They are present in abundance at infection sites to which they are attracted by chemotactic compounds from injured cells.

2.8 Measures of immunity

The commonly used and assessed method in poultry for measuring immune response are lymphoid organ weights, [35] antibody response to foreign antigens, [36] heterophil: lymphocyte (H:L) ratios [37] and lymphocyte blastogenesis assays [38]. Zulkifli and Siegel (1995); Borges (1997) reported that H/L ratio has been indicated to be a good quantitative measure of stress.

Lymphoid organ (Bursa, thymus, and spleen) weights are easily measured and reflect the body's ability to provide lymphoid cells during an immune response. Suppression of immune response may be due to temporary or permanent damage to primary lymphoid organs which results in increased susceptibility of the host of various bacterial, viral and parasitic infections [39].

2.9 Effect of Vitamin C on immune response

The role of ascorbic acid as mentioned earlier by Nockles and Schemlibg (1978) was found to reduce the amount of corticosterone in the plasma with subsequent maintenance of the normal leucocytes count.

In the first line of defence against pathogens, phagocytosis by neutrophils involves increased consumption of both ascerbate and dehydroascorbate [40]. Ascorbic acid can modulate the activity of B cells and addition of dietary ascorbate prior to immunization has been found to increase antibody production [41]. Their findings indicate that the cell mediated immunity and the antibody response to sheep red blood cells were not altered by the dietary supplement of 10 g ascorbic acid kg. They also observed that ascorbic acid supplementation increase the heterophil lymphocyte (H/L) level from 4 to 6 days post-vaccination. Freeman (1986) suggested that the mechanism by which ascorbic acid ameliorates the steroid mediated immuno-suppression to be either by reducing adrenal synthesis of corticoids or by protecting the lymphoid tissue. Dietary supplementation with vitamin C, therefore, may have beneficial effects on immuno-responsitiveness in chicken.

Pardue and Thaxton, (1984) found that supplementation of broiler with vitamin C has ameliorated steroid mediated Immune suppression. furthermore, [41] found a significant reduction in adrenal size in vitamin C supplemented chicks this finding led the authors to suggest that vitamin C suppresses adrenocortical synthesis in the chicken.

Freeman, (1986) found that many organs (Spleen, liver and intestine) were shown to contain a concentration of ascorbic acid several times greater than that of the plasma.

2.10 Effect of dietary vitamin C on Immune response of heat stressed broiler

A report by Thaxton (1986) showed that, following infection with infectious bursal disease (IBD) virus, vitamin C protected the immune biological tissues in growing birds and reduced their mortality to infection in a hot environment. Furthermore, [41]. showed that immunosuppression at high environmental temperatures could result from a reduction in thyroid activity.

Therefore, it may be that the reduction of weight loss of an immunocompetent organ induced by feeding ascorbic acid may be associated with thyroid activity.

Farooq (2005) observed that betafin and vitamin C can improve immunity of bird during heat stress. He found that best titter was in group without heat stress followed by group supplemented with vitamin C and the lost titter in group with heat stress.

Vitamin C decreases the corticosterone in blood circulation which increases during exposure to ambient temperature as a demonstrated by Richard (1998). This led to decrease in the lymphocyte's percentage and according by the total white blood cell increased.

CHAPTER THREE

RESULTS

3.1. Feed intake

The effect of different levels of ascorbic acid on weekly feed consumption is shown in table (1). The obtained result indicates that there was no significant different in the first three weeks in the feed intake between the different treatments. However, in the fourth week the feed intake was significantly ($p \le 0.05$) high in the groups fed diet supplemented with low level (150mg/kg) and moderate level (350mg/Kg) of ascorbic acid compared to those fed (550mg/kg). In the fifth and six week there was no significant ($p \ge 0.05$) difference in feed intake between the birds fed (0,150,350mg/kg). However, significant ($p \le 0.05$) lower feed intake was observed in bird fed diet supplemented with the higher level of ascorbic acid (550mg/Kg).

3.2 Body weight gain

The means of weekly weight gain of experimental birds during six weeks of age is presented in table (2). In the third and six week of age there was significant difference ($p \le 0.05$) in the weight gain of the bird fed diet supplemented with 350mg/kg where high weight gain was obtained compared to other treatments. In contrast, in the fourth and fifth week there was no significant difference ($p \ge 0.05$) in the weight gain of the bird fed diet supplemented.

3.3 Feed conversion Ratio

Data shown in table (3) represent the feed conversion ratio of bird reared during summer and fed different level of ascorbic acid. The response of the experimental birds showed significant difference ($p \le 0.05$) from the third week of age but there was no significant difference in the first two weeks. In the third, fourth and fifth week the feed conversion ratio was significantly ($p \le 0.05$) low in the group fed diet supplemented with (350mg/kg) ascorbic acid. In the six weeks of age their lower feed conversion ratio was observed in bird fed diet supplemented with 150mg/kg and 350mg/kg.

Table 1: Effect of different levels of ascorbic acid on weekly feed intake of broiler chicks (g/bird).

	Lev		ic acid (mg/I	•6)	
Weeks	0	150	350	550	SEM
Week3	390.75	417.75	385.25	379.50	16.583 ^{N.S}
Week4	457.25 ^{ab}	488.75ª	492.75 ^a	438.75 ^b	24.663**
Week5	590.25 ^ª	601.50 ^a	614.00 ^a	557.25 ^b	12.774**
Week6	649.25 ^a	664.00 ^a	665.75ª	613.25 ^b	32.701**

Means in the same row with the same letter are not significantly different.

- **= statistically significant ($p \le 0.05$)
- N.S = Not statistically significant.

SEM = Stander error of the means.

Table 2: Effect of different levels of ascorbic acid on weekly Weight gain of broiler chicks (g/bird) reared at high temperature.

Levels of ascorbic acid (mg/Kg)						
Weeks	0	150	350	550	SEM	
Week3	201.07 ^{ab}	195.29 ^{ab}	220.00 ^a	186.93 ^b	27.765**	
Week4	200.00	185.72	190.72	180.72	22.779 ^{N.S}	
Week5	300.36	295.53	305.60	276.07	44.106 ^{N.S}	
Week6	230.71 ^b	330.36 ^a	341.73 ^a	230.36 ^b	64.153**	

Means in the same row with the same letter are not significantly different. **= statistically significant ($p \le 0.05$)

N.S = Not statistically significant.

SEM = Stander error of the means.

Table 3:Effect of different levels of ascorbic acid on weekly feed conversion
ratio of broiler chicks (g feed/g body weight) during summer.

	Leve	els of ascorbi	c acid (mg/K	g)	
Weeks	0	150	350	550	SEM
Week3	1.9600 ^{ab}	2.1675 ^a	1.7500 ^b	2.0375 ^{ab}	0.121**
Week4	2.7625ª	2.6675ª	2.4750 ^b	2.7625 ^a	0.439**
Week5	2.0475 ^a	2.1200 ^a	1.9375 ^b	2.0925 ^{ab}	0.164**
Week6	2.8500 ^a	2.1850 ^b	2.0875 ^b	2.7050 ^a	0.245**

Means in the same row with the same letter are not significantly different.

**= statistically significant (p≤0.05)

N.S = Not statistically significant.

SEM = Stander error of the means.

Table 4: Effect of different levels of ascorbic acid on lymphoid organs of broilers (g/bird).

	Bursa	Thymus	Spleen	BBWR	TBWR	SBWR
0	0.8313±0.37	3.5363±1.03	0.8463±0.37	0.069±0.03	0.29±0.08	0.07±0.03
150	0.6125±0.17	3.3375±1.59	0.6775±0.27	0.049±0.02	0.29±0.13	0.05±0.02
350	0.7013±0.26	2.8263±1.26	0.6512±0.19	0.05±0.02	0.22±0.10	0.05±0.01
550	0.6075±0.23	3.4613±1.23	0.6125±0.19	0.05±0.02	0.29±0.10	0.05±0.02

Means in the same row with the same letter are not significantly different ($p \le 0.05$).

BBWR= Bursa/body weight TBWR= Thymus/body weight SBWR= Spleen/body weight

Chapter Four

4.1 CONCULUSION

This study indicated that ascorbic acid supplementation during heat stress had beneficial effects on weight gain, FCR, and serum antibody development of heat-stressed birds.

It is concluded from this experiment that the ascorbic acid has expressed itself as an anti-heat stressor and an immune potentiating agent under heat stress conditions.

4.2 RECOMMENDATION

Ascorbic acid can be used to minimize stability of the bird to viral disease since it increases antibody titer agents Newcastle virus It can be recommended that vitamin C can be used during high temperature as feed additives with level less than 500mg/Kg to alleviate the negative effect of high temperature More researches needed to evaluate its effect when it combined with other vitamins and its effect on bacterial disease.

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يوخته

ترشى ئەسكۆربىك كەبە شېرەيەكى بەرفراوان بە ۋېتامىن سى ناسراوە، ماددەيەكى خۆراكى سەر مكييە بۆ ئاز ملەكانى و مك يەلەو ەر. ترشى ئەسكۆر بېك لە خۆر اكى يەلەوەر دا تەندر وستى ئاژ ملهکان باشتر دمکات و بهم شبو میه تو انای گهشهکر دنی بالندهکان زیاد دمکات. تر شی ئهسکو ربیک دەتوانرېت بە شېوەي بەر ھەمى دەستكرد بەكاربەينرېت يان بە شېوەيەكى سروشتى لە مىوە و ر و و مكهكانه و م بدهستبهينريت. له ناودا دهتويته و و به ناساني دهتو انريت له ناوي خو اردنه و و خۆراكدا بەكاربەينريت. يەلموەر دەتوانىت ترشى ئەسكۆربىك لەجەستەدا دروست بكات. بەلام دەتو انرېت ئەداى ئاژ ەللەكان باشتر بكرېت بە زېادكر دنى ترشى ئەسكۆر بېك بۆ خۆر اكەكانيان. جگە لمو مش ترشى ئەسكۆر بېك بېلى دەوترېت دژە ئۆكسېنەر و دژە ھەركردن. ئەمەش بەرگريان بەر امبەر نهخوشی زیاد دهکات له و مرزی گو استنه مدا. ته و او که ری ترشی ئه سکور بیک کاریگه ری ئه پنی لمسمر وه لامي فشار دهبيت، بهتايبهتي له وهرزي وشكهساليدا له و لاتاني گهرمدا. جگه لهوهش تهواوكهري ترشى ئەسكۆر بېك له خۆر اكى بەلەو ەردا بەرگرى بەر امبەر نەخۆشيەكان باشتر دەكات و فشار ريكدهخات و يارمهتي برۆسەي ئۆكساندنى جەستە دەدات. لەكۆتايىدا ئەمە ريزەي ھىلكەدان و توانای هیلکهکردنی هیلکه و بهر هممهینانی بهرزتری پهلموهر بهرز دهکاتموه. بز چینهکان له کوتایی ماو می هنلکهدانهکهدا بار مهتیده ره بق زیادکردنی کو النتی تو نکلی هنلکه و ریز می شکاوی هنلکه و E كەمدەكاتەرە. ترشى ئەسكۆربىك يەيوەندىيەكى بەھىزى لەگەل ۋىتامىنەكانى ترى وەك ۋىتامىن ماددهکانی تری و هک زینک و زهیتی زهیتی ز هیتوون و ترشی فولیک و خوراکی ریشالی ههیه. ئهم ييداچوونموهيه ئامانجيهتي همموو زانيارييهكاني ترشي ئەسكۆربيك له خۆراكى يەلموەردا كۆبكاتموه، بەمەش رۆلى كشتى ترشى ئەسكۆربىك بۆ يېشەسازى يەلەرەر دابېن بكات