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Effect of folic acid and cobalt on milk yield and its components of ewes and their growing lambs

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This experiment was conducted on 20 heads of pregnant Awassi ewes with sucking lambs in one of the private farm in AL- Fayadhia village, 7-10 km from Al-Qassim city to the east of Hillacity. This study was deals with two trials .The first experiment, the animals was random distributed into four treatments. The first treatment (control) without folic acid and cobalt. The second treatment was used 5 mg of folic acid for each ewe per day. The third treatment was used with 80 µgm of cobalt for each ewe and twice every week and fourth treatment, the ewes were received combination of folic acid and cobalt as the same concentrations above. The second experiment was started from the date of lambs' birth and continued till the age of weaning (90 days).The lambs was distributed randomly to four transactions, the first treatment was called control group and gave the second treatment of animals with 0.5 mg of folic acid to twice a week. The Animals of third treatment were treated with 40 µgm of cobalt with twice for a week. The fourth treatment was mixed with folic acid and cobalt in the same concentrations. The results of ewes showed high significant differences (P≤0.01) in average weights of ewes at the end of gestation, at birth, at weaning, daily feed intake, food conversion efficiency, daily milk production and proportions of components except Solids Non Fats (SNF) and concentrations of cholesterol, triglycerides and blood proteins which it was in favor of experimental treatments that containing folic acid and cobalt compared to the control treatment. The results of Awassi lambs showed high significant differences (P≤0.01) in means of initial weight at birth, final weight at weaning , feed consumption, milk intake, cholesterol ,triglycerides and blood proteins which were for benefit of the experimental groups which contained folic acid and cobalt compared to the control treatment. We conclude from results of this study that dosage of animals with folic acid and cobalt has been led to improve the health status as a result of significant improvement in productive performance, growth and blood characteristics of ewes and lambs compared to the control treatment.

Keywords: folic acid, cobalt, milk yield, milk ingredients, blood proteins.

INTRODUCTION

The sheep are an integral part of the livestock sector and of the national income sources in Iraq, The population of sheep in Iraq was about 6,200 million for year 2006 (Arab Organization for Agricultural Development, 2007).The researchers and workers (AL-Sayegh and AL-Kass,2006) in private and mixed sector projects have been looking for important ways to improve the productive and reproductive efficiency of local sheep and fattening of farm animals, such as giving vitamins to animals in many methods, including directly with food, dosage of mouth or injections under the skin. The food additives used in this experiment were folic acid (vitamin B_9) and cobalt to meet the nutrient requirements for maintenance, growth, reproduction and vital functions in the body and also to overcome of nutritional deficiency of folic acid and cobalt or both of them which leads to Anemia disease (AL-Chalabi and Ezz AL-Din,

1982). It must be providing and securing nutrient requirements in growth and maintenance diets of pregnant ewes and developing lambs during the growth and fattening period (El-Barody, 2002). The folic acid is known as vitamin B_9, is one of the water soluble vitamins (B-plex),which is essential for safety and maintenance of pregnancy and growth of infants because its vital role in synthesis of DNA and RNA (Davis and Nicole,1988) and synthesis of protein tissues in the body of developing animals (Kolb., 1999).This is due to that infant animals cannot synthesize folic acid in their rumen (Duseski et al.,1996).The results of some studies indicate that giving folic acid to small animals for an age of 2-18 weeks was led to improving their growth (Dumoulin et al., 1996).So as Kolb et al, (1999) indicates that folic acid was contributes to building and regeneration of cells in the body tissues of the animal as result of the division of cells and thus leads to increased rapid body growth of developing animals this one hand and on the other hand most of studies have indicated that the animals was treated with folic acid was leads to significantly improved in rates of daily weight gains and percentage of vitality of animals when compared to animals which not treated with this vitamin. Also observed a positive relationship between vitamin B_9 (folic acid) and vitamin C (Ascorbic acid).The increase in level of folic acid in the blood which leads to an increase concentration of vitamin C, which works to protect the cellular membrane from the self-oxidation which contribute to modification of temperature resulting from nutrient metabolism which leads to a condition called thermal regulation inside of animal body was treated by folic acid and thus improves of food conversion (Kolb et al.,1999; El-Barody, 2002, and Almahdawi et al., 2009). Wasfi (2009) noted, eating of combination of folic acid with vitamin B_{12} are prevents clotting and atherosclerosis due to reduced homocysteine in the blood when the homocysteine levels in blood suddenly increase, leading to heart strok. Results of Girard, Matte (2005) and Graulet et al., (2007) showed that folic acid supplementation with vitamin B_{12} by intramuscular injection of milking cows was resulted significant increase in milk yield and its components, such as protein of milk, milk fat, and lactose sugar than compared to control group. In the same vein, Preynat et al., (2009) observed significant differences in milk protein, lactose (milk sugar) and total solids ratios in dairy cows groups that were given vitamin B_9 by intramuscular injection compared to control group.As well as AL-Zubaidy and AL-Taey,(2012) showed significant differences in rates of daily and total weight gains between the lambs groups which were treated with folic acid and vitamin A than for control group. Also Almahdawi (2018) showed significant improvement in averages of daily milk production and the proportions of its components of ewes groups which were given folic acid when compared to the control group.

Cobalt is a chemical element and symbolized is (Co), and it is an antioxidant that promotes immunity (Puls, 1994). Therefore it is considered necessary to provision in diets of pregnant sheep. Moreover, it contributes to the manufacture of vitamin B_{12} in the rumen and has an additional role to producing of vitamins B –plex specially as thiamine and nicotine acid. It also works on regeneration and synthesis of red blood cells. Cobalt deficiency to causes a severe decline in growth, diarrhea, falling wool, depression of milk yield, anemia, metabolic disorders, elevated concentrations of AST and ALT enzymes, and inability to represent propionic acid (Puls , 1994 and Akins, 2012). Kadim et al., (2003) noted that lack of cobalt in diets of pregnant ewes to causes decrease in number of microorganisms in the rumen, which was resulting to decrease food digestion in the rumen. In this direction, Boland et al., (2008) observed significant differences in averages of lambs weight at the time of birth and the amount of milk consumed which were in favor of lamb group treated with the cobalt when using the two rations the first without cobalt and second had contained cobalt in lambs ration during the suckled period. Akins, (2012) was found significant differences in milk yield and percentage of protein, and non-significant differences in food conversion efficiency, milk fat and lactose percentages of dairy cows (Holstein) groups that were treated by cobalt compared to control group. These results was confirmed with Abou-Zeina et al.,(2008) who observed a significant improvement in means of total protein, albumin and globulin concentrations in blood of Egyptian sheep treated with cobalt in ration compared to the group of sheep that ate low level of cobalt. Quirk and Norton (2010) noted no found significant differences in amounts of milk produced, total solids, protein, and fat in milk of heifers which treated with cobalt in the diets when compared to control group (without cobalt).Due to the synergistic and functional role and close interaction in mechanism and joint functions of folic acid and cobalt as antioxidants and their role to improving the productive and reproductive performance in local sheep. Therefore, the aimed of present study to study the effect of folic acid and cobalt on milk yield and its composition of ewes

and to improve the health status, safety and maintenance of pregnancy and to obtain very active and vital lambs without congenital malformations through their synergistic effect to improving of productive performance and some physiological and biochemical properties of blood.

MATERIALS AND METHODS

This study was conducted in private farming AL-Fayadhia village which is about 7-10 km from Al-Qassim city to the east of Hilla city, Babylon Province.

This study included two experiments:

1.The first experiment:

Twenty of ewes were used during the experimental period. They were fairly homogeneous in live weight and age. They age wereabout2.5-3 years. The initial weight was 35-36 kg and health status of the animals was thin in appearance. These ewes were placed under monitoring and follow-up periodically by giving them dietary payment for two weeks before starting of this experiment. All ewe s were vaccinated with Rafoxanide twice at the beginning of the trial period and second doses were after three weeks in order to killing liver worms. The ewes were then randomly distributed into four treatments at rate of 5 heads per treatment. The first treatment was considered control group without folic acid and cobalt, the second treatment, ewes were giving 5 mg of folic acid / ewe per day. In the third treatment, the ewes were giving 80 micrograms of cobalt/ewes for twice to week. In the fourth treatment, animals were given combination of folic acid and cobalt as the same concentrations above during the experiment.

The groups of ewes were fed ad libitum and group feeding of daily diets. The first daily meals were served at 8 am and the second at 5 pm. As for the remaining fodder, was collected and then weighed and subtracted from the amount of feed provided for calculating the amount of daily feed consumption. In addition, the sheep was obtained the coarse feed through daily grazing for five hours on the short grass available in the pastures throughout the experiment. Data of ewes were taken during pregnancy, birth and weaning periods which included were ewe's weight every two weeks, amount of feed intake, food conversion and amount of daily milk product. The milk production was started recording at fourth day after birth and continued this process once per week until lambs weaning where isolating of lambs from her ewes at evening and the next morning are milking the ewes by manually method and recording amount of milk per ewes and then let the lambs with mothers for 20 minutes to obtain the remaining milk from udder. The amount of milk intake was calculating by the difference in lambs weight before and after the lactation, but this process is repeated morning and evening once a week (Hadijpanyiotou and Louca, 1976). Milk samples were taken by manual milking of each ewe before suckling the lambs from their mothers and another sample of milk was taken after the lactation process. The two samples were then mixed to obtain homogeneous sample of milk to overcome the problem of different proportions of the milk components. The chemical analysis of milk samples was carried out in the nutrition laboratory of the Public Health Branch of the Faculty of Veterinary Medicine/Al-Qassim Green University to estimate the proportions of milk components. In the last week of pregnancy, 10 ml of blood samples were pulled from the jugular vein. Serum was separated from the thrombosis by using centrifuge at 3000 rpm for 15 minutes. The samples were then putted in plastic tubes for biochemical tests to measure concentration of total protein by method (Green and Clark, 1982), albumin by Bush (1998), cholesterol (Allain et al., 1974) and triglycerides by Tietz et al., (1999).As for globulin, it was calculated by difference between total protein and albumin according to Otto et al., (2000).

2.Second experiment:

This experiment was conducted on 20 newly lambs of ewes which used in the first experiment. The lambs were randomly distributed to four treatments at rate of 5 lambs per treatment. The first treatment is considered a control treatment and the second treatment of lambs were treated with 0.5 mg of folic acid (vitamin B_9) for twice times per week. The third treatment of lambs was giving of 40 micrograms of cobalt/lamb to twice a week .The animals of fourth treatment were combination of (B_9 + Co) with the same concentrations mentioned above the dosages initiated by using the listed veterinary system after four hours of lambs' birth. The procedure was continued from the date of birth until they reached the age of weaning (90 days). During this experiment, the weight of the lambs was measured immediately at birth, the age of weaning, and calculated the average of total and daily weight gains, amount of milk consumed and the amount of daily feed intake. Blood samples were withdrawn by 10 ml from jugular veins at the end of the trial period to measure total protein, albumin, globulin, cholesterol and triglycerides as

in the first experiment. The statistical analysis of the data on ewes and lambs in both experiments was performed according to the complete random design (AL-Rawi and Khalaf Allah,2000) to determine the effect of folic acid and cobalt on the production performance and some blood characteristics of pregnant Awassi ewes and their lambs .

The mathematical model was used as follows:

$Y_{ij} = \mu + t_i + e_{ij}$

Y_{ij} = observation value (j) at treatment (i).

μ = the overall average of all observations.

t_i = Effect of treatment (i) which represents the effect of folic acid and cobalt on the productive performance and some blood characteristics of pregnant ewes and their lambs .

e_{ij} = Random experimental error of the experimental unit, which is distributed naturally and independently at a mean of zero and variance of $\sigma^2 e$.

Duncan test (AL-Zubaidy and AL-Falahy, 2018) was conducted at probability level of 5% or 1% to examine the significance differences between the averages of the studied traits using by SAS program (2012).

RESULTS AND DISCUSSION

The results of this study included two experiments to study the effect of dosage with folic acid and cobalt on the following characteristics:

1. Productive traits of ewes and lambs.

The results of the statistical analysis in table (1) showed convergence in initial weight of the ewes at the beginning of experiment. This indicates complete homogeneity and removing of individual differences between the groups of ewes that were under study. The initial weight of ewes at the beginning of experiment was 35.90, 36.14, 36.36 and 36.62 kg of four groups respectively. The results of the statistical analysis showed that there were high significant differences ($p \leq 0.01$) on final weight at the end of pregnancy between both second, third treatments than for the first and fourth treatments, as well as the results were mentioned in table (1) the presence of highly significant differences ($P \leq 0.01$) in averages of ewes weight at birth and at weaning, amount of daily feed intake and feed conversion efficiency between the first, second than for fourth transactions. The average of final weight of pregnant ewes at the end of gestation was 47.50, 49.28, 49.62, 50.98 kg, and mean of ewes weight at birth 42.30, 44.64, 45.42, 46.24 kg and rates of ewes weight at weaning were 43.78, 46.64, 47.32, 47.88 kg, daily feed intake was 1.549, 1.633, 1.726, 1.745 kg and food conversion efficiency 4.25, 3.58, 3.24, 3.19 kg Fodder/kg milk produced of four treatments respectively.

Table 1.Effect of folic acid and cobalt on the productive performance of Awassi pregnant ewes.

Traits	First treatment (control)	Second treatment (folic acid)	Third treatment (cobalt)	Fourth treatment (folic acid + cobalt)
1.No. of ewes :	5	5	5	5
2. Initial weight. (kg) NS	35.90 ± 0.30 A	36.14 ± 0.24 A	36.36 ± 0.50 A	36.62 ± 0.54 A
3.The weight at the end of pregnancy. (kg)**	47.50 ± 0.39 C	49.28 ± 0.38 B	49.62± 0.22 B	50.98 ± 0.35 A
4. The weight at birth. (kg)**	42.30 ± 0.42 C	44.64 ± 0.36 B	45.42 ± 0.17 AB	46.24 ± 0.50 A
5. The wright at weaning. (kg)**	43.78 ± 0.55 C	46.64 ± 0.37 B	47.32 ± 0.19 AB	47.88 ± 0.29 A
6.Daily feed intake.(kg)**	1.549 ± 0.01 C	1.633 ± 0.02 B	1.726 ± 0.02 A	1.745 ± 0.02 A
7.Feed conversion (kg of feed /kg of milk) **	4.25 ± 0.10 A	3.58 ± 0.14 B	3.24 ± 0.09 BC	3.19 ± 0.12 C

The averages of traits which have carried different letters horizontally indicate significant differences at 0.05 or 0.01.
NS = Non Significant. *: Significant differences at 0.05. **: high significant differences at 0.01.

The significant superiority of final weight of the ewes in the last three treatments containing folic acid, cobalt and mixed of them compared to the control treatment because of activity effectiveness of folic acid and cobalt and their synergistic role in formation and synthesis of nucleic acids (RNA and DNA) and thus positively reflected in deposition of protein tissues of all body tissues as a result of increasing efficiency of absorption of amino acids in the small intestine after transported microbial

protein and part of food protein from the rumen and the activation of metabolic processes of food to cover the needs of the maintenance and pregnancy of pregnant ewes which has been positively reflected in improving of pregnant performance of animals (Pour, 2010, Akins, 2012 and Pereria et al., 2013).On the other hand, it may be to improvement in final weight of ewes because to increase amount of feed intake for ewes groups which treated with folic acid and cobalt, which led to activation of metabolic processes and thus to meet the needs of the ewes and fetus from nutrient compounds at the end of pregnancy and thus reflected positively to increase live weight at the end of pregnancy. Noted of results in table (1) high significant improvement (P≤0.01) in feed conversion efficiency in favor of experimental treatments of ewes that treated with folic acid and cobalt than for control treatment due to the effectiveness and activity of folic acid and cobalt which leads to increasing number of microorganisms in rumen which led to activate the process of absorption of amino acids in the small intestine after transference of microbial protein and a part of the food protein from the rumen, which contributed to activation of nutrient metabolism in the cells and tissues of the body and thus reflected its effect to improving of feed conversion efficiency in order to cover their nutrient requirements for maintenance, growth and pregnancy during pregnancy period (Matte et al.,1990 and Kolb,1999).This results was consistent with results of AL-Zubaidy and AL-Taey, (2012) who showed significant differences in rates of daily and total weight gains between the lambs groups which were treated with folic acid and vitamin A than for control group of lambs. These results were consistent with Almahdawi (2018), which found high significant differences in average of final weight of ewes at the end of pregnancy and at birth, daily feed intake and feed conversion efficiency in favor of folic acid and vitamin B_{12} and their combinations of them than for control treatment. This results were not consistent with results of Graulet et al., (2007), who observed no significant differences on dry matter intake between four treatments which were the first was considered control treatment and the second contained folic acid, the third contained vitamin B_{12} and the fourth contained a mixture of (folic acid + cyanocobalamine) in feeding of milking cows. As for the second experiment of this study, the results presented in table (2) were appeared high significant effect (P≤0.01) of folic acid and cobalt were given to all ewes during pregnancy period on lambs weight at birth , between the first treatments than other treatments which reached 4.04, 6.10, 6.06 and 6.45 kg respectively. The results shown in table (2) high integer superiority (P≤0.01) in means of live weights of lambs at birth to benefit of experimental groups when compared to the control group due to the effective role and synergistic coordination for combined of folic acid and cobalt to improve vital function and regenerating speed of cellular division due to the increased concentration of folate in blood serum of the pregnant mother, which is involved in construction and synthesis of DNA and RNA and deposition of protein tissues in all body tissues of pregnant ewes and thus reflected to increase lambs weights at birth (Pour, 2010 and Pereria et al.,2013).This results was agreement with mentioned of El-Barody (2002), who found account improvement in mean of growth rates of ossimi lambs which was treated with folic when he was using different levels of folic acid (0.30, 0.60 mg / kg live weight) when compared to the control group of lambs. In the same context, the results presented in table (2) showed high significant effect (P≤0.01) of folic acid and cobalt on rates of final weight at weaning, daily and total weight gain between both second ,third treatments than for first and fourth treatments .On the other hand between the first and fourth treatments. The final weights of lambs at weaning were 21.86, 24.82, 25.50, 28.50 kg and the daily weight gains 198, 208, 216, 245 gm/day and the total weight gains 17.82, 18.72, 19.44, 22.05 kg of four transactions respectively. The results showed high significant differences (P≤0.01) between both first, second treatments than for third and fourth treatments in average of daily amount of milk consumed .While there were no significant differences in mean of daily feed intake between four treatments. The average of daily amount of feed intake was 426, 438, 454, 474 gm/feed/day and daily milk intake 361, 450, 510, 530 gm milk/lamb/day for four treatments respectively.

Table 2. Effect of folic acid and cobalt on the productive performance of Awassilambs .

Traits	First treatment (control)	Second treatment (folic acid)	Third treatment (cobalt)	Fourth treatment (folic acid + cobalt)
1.No. of lambs :	5	5	5	5
2.The weight at birth (kg)**	4.04 ± 0.11 B	6.10 ± 0.22 A	6.06 ± 0.23 A	6.45 ± 0.16 A
3.The final weight (kg)**	21.86 ± 0.21 C	24.82 ± 0.33 B	25.50 ± 0.49 B	28.50 ± 0.14 A
4.Daily weight gains (gm)**	198 ± 1.08 C	208 ± 4.71 BC	216 ± 7.51 B	245 ± 2.15 A

5.Total weight gains (kg).**	17.82 ± 0.11 C	18.72 ± 0.43 BC	19.44 ± 0.68 B	22.05 ± 0.19 A
6. Daily feed intake (gm)NS	426 ± 10.77 A	438 ± 5.68 A	454 ± 9.62 A	474 ± 29.27 A
7. Daily milk intake (gm)**	361 ± 9.03 C	450 ± 17.68 B	510 ± 20.19 A	530 ± 8.93 A

The averages of traits which have carried different letters horizontally indicate significant differences at 0.05 or 0.01.
NS = Non Significant. *: Significant differences at 0.05. **: high significant differences at 0.01.

The results showed a positive and high significant improvement on growth of lambs in favor of folic acid and cobalt treatments when compared with control group. This was due to significant increase in daily consumption of milk and fodder obtained by the experimental groups that were treated with folic acid and cobalt compared with the comparison lambs group. These results were appeared in table (2) significant improvement in the average of daily milk intake for groups treated with folic acid and cobalt compared with the control group. This is due to increase in daily and total weight gains of the lambs body, which is offset by daily increase in feed intake of fodder and milk to cover the maintenance and growth needs of lambs. This results were consistent with result of Dupplesis et al., (2014) who observed a significant decrease in daily of dry intake which was 80.4 kg in dairy cows group (B9 + B12) versus 76.6 kg for control group for the milk season of 134 days. Also the results indicate a significant improvement on rates of daily and total weight gain of the experimental groups which treated with folic acid and cobalt compared to the control group. The reason is due to synergistic activity between folic acid and cobalt may led to stimulated the regeneration of cell division and protein deposition in all tissues of the body of lambs (Davis and Nicol 1998, Kolb et al 1999, Quirk and Norton, 2010) . The results of this study were consistent with those of Kolb et al., (1999), El-Barody (2002) and Almahdawi et al., (2009) who they found significant improvement in rates of daily weight gain when they using different percentages of folic acid in diets of ruminants. Also the results were consistent with Almahdawi (2018), who observed a significant improvement in the amount of milk intake in favor of experimental groups which were treated with folic acid and cobalt when compared to the control group. The results did not agree with Preynat et al.,(2009) who did not noted statistically significant differences in live body weight when using two rations, the first group is called the control group and the second contains 16 mg of folic acid and 10 mg of vitamin B12 in milk nutrition.

2.Milk yield and its compositions .

The results in table (3) showed high significant differences (P≤0.01) between the third, fourth treatments than for the first and second treatments in average of daily milk production and percentages of protein and ash in samples of milk sheep, it is one hand and another hand, it was found high significant differences (P≤0.01) between the first of three treatments than for last treatment in averages of moisture, fat of milk, lactose sugar and total solids. but there were no significant differences between four treatments on percentage of Solids Non Fats (SNF) in milk samples of Awassiewes. The average of daily milk production was 365,459,533,549gm milk/day, percentages of milk protein were 4.23, 4.34, 4.48, 4.59%, ash 0.95, 0.90, 0.88, 0.85% and moisture content 86.17, 85.50, 85.10 and 83.81%, milk fat 4.41, 4.86, 5.21, 6.43%,lactose sugar 4.24, 4.38, 4.45, 4.89%, solids non fats (SNF) 9.42, 9.63, 9.69, 9.76% and total solids (TS) 13.83,14.49,14.90 and 16.19% of four treatments respectively. This result was in favor of the experimental treatments that containing folic acid, cobalt and mixture between of them (B9 + Co) when compared to control treatment. The results presented in table (3) showed high significant differences in protein percent in favor of experimental groups that were treated with folic acid and cobalt when compared to control group due to the structure and activity of folic acid and cobalt, which act as coenzymes to stimulate nutrient metabolism and building of protein tissues as result of increasing of feed intake and improved utilization of the diet protein , which is absorbed in the small intestine to produce amino acids and thus directly contributes to raising proportion of milk protein (Almahdawi, 2018).

Table 3. Effect of folic acid and cobalt on the milk yield and its components of Awassiewes .

Traits	First treatment (control)	Second treatment (folic acid)	Third treatment (cobalt)	Fourth treatment (folic acid + cobalt)
1.No. of samples;	5	5	5	5
2.Dailly milk yield (gm)**	365 ± 9.63 C	459 ± 17.68 B	533 ± 21.94 A	549 ± 16.65 A
2.Moistture (%).**	86.17 ± 0.05 A	85.50 ± 0.24 B	85.10 ± 0.19 B	83.81 ± 0.16 A
3.Crude protein (%).**	4.23 ± 0.03 C	4.34 ± 0.07 BC	4.48 ± 0.08 AB	4.59 ± 0.04 A
4. Ether Extract (%).**	4.41 ± 0.06 C	4.86 ± 0.21 BC	5.21 ± 0.23 B	6.43 ± 0.17 A

5.Lactose (%).*	4.24 ± 0.02 B	4.38 ± 0.08 B	4.45 ± 0.03 B	4.89 ± 0.25 A
6.Ash (%).**	0.95 ± 0.01 A	0.90 ± 0.02 B	0.88 ± 0.01 AB	0.85 ± 0.02 C
7.Soluble Non Fats (%).NS	9.42 ± 0.02 A	9.63 ± 0.14 A	9.69 ± 0.11 A	9.76 ± 0.16 A
8.Total Solids (TS) (%).**	13.83 ± 0.06 C	14.49 ± 0.24 B	14.90 ± 0.19 B	16.19 ± 0.16 A

The averages of traits which have carried different letters horizontally indicate significant differences at 0.05 or 0.01.
NS = Non Significant. *: Significant differences at 0.05. **: high significant differences at 0.01.

Another reason may be due to increase size of the gastrointestinal tract as a result of optimizing utilization of fodder consumption for ewes treated with folic acid and cobalt, which led to increase in size of the lactation gland of ewes, which was reflected to increase ratio of milk protein (AL-Sayegh and AL-Kass, 2006).We noted significant increase on ratio of milk fat for the sheep groups were treated with folic acid and cobalt compared to the control group, due to both of folic acid and cobalt were acting as enzymatic agents to increase activity of nutrient metabolism and construction of new protein tissues to replacing of damaged tissue and leads to increasing the concentration of acetic acid in the rumen, which plays role by increasing of milk fat (AL-Sayegh and AL-Kass, 2006).This results were consistent with Girard et al.,(2005),who observed significant differences in milk production and lactose sugar of dairy cows groups were treated with vitamins than for control group when using four diets containing different levels of folic acid/ kg in rations of dairy cattle (Holestein) for milk season (305 days).Also this results were consistent with those of (Girard and Matte 2005), who found significant differences in daily of milk production, solids non fats, lactose sugar, ash, and total solids among four treatments ,the first was control (without vitamins), the second treatment was (B_9), the third treatment was (B_{12}), and fourth treatment had contained combination of ($B_9 + B_{12}$) in feeding of the Holestein cows for 305 days period. In the same way, this results are consistent with the results of (Quirk and Norton 2010), who observed significant increase in average of daily milk produced, protein, fat and total solids of milking heifers which was treated with cobalt compared to the control group. Also this results were consistent with the results of Akins (2012), which found significant differences in rates of daily milk yield and percentages of protein, fat and lactose between three levels of cobalt (low, medium, high) in rations of dairy cows. The results were consistent with results of Almahdawi, (2018), which observed a significant improvement in average of daily milk production and proportions of its components in ewes groups which treated with folic acid and vitamin B_{12} compared to the control group. This results were not consistent with results of (Kristen et al., 2003) who found non-significant on daily of milk yield and percentages of protein, fat and lactose in milk samples when they were using different levels of cobalt (0.13, 0.20, 0.27 mg / kg dry matter) of milking cows. The results of this study were not consistent with the results of Graulet et al., (2007) who observed no significant differences on milk protein, milk sugar (lactose) and total solids between four rations which were (control ,vitamin B_9, vitamin B_{12}, $B_9 + B_{12}$) in rations of milking cows.

3. Blood parameters:

The results of the statistical analysis appeared in table (4) high significant differences (P≤0.01) between the third, fourth treatments than for the first and second treatments in concentrations of total protein, and globulin of pregnant ewes. While observed high significant differences (P≤0.01) between the first treatments when compared to the last three treatments in albumin concentration. The concentrations of total protein were 3.66, 5.46, 5.96, 6.16 gm/dl and albumin 1.36, 2.36, 2.48, 2.60 gm/dl and globulin 2.30,3.10,3.48,3.56gm/dl in blood serum of Awassi ewes respectively. The results indicate higher significantly in concentrations of total protein in favor of experimental treatments when compared to control treatment.

Table 4. Effect of folic acid and cobalt on concentrations of cholesterol, triglyceride and blood proteins of Awassiewes .

Traits	First treatment (control)	Second treatment (folic acid)	Third treatment (cobalt)	Fourth treatment (folic acid + cobalt)
1.Cholesterol (mg)**	159.14 ± 2.71 A	153.04 ± 1.46 B	142.00 ± 1.02 C	124.78 ± 2.03 D
2. Triglycerides (mg).**	68.26 ± 0.88 A	60.03 ± 2.29 B	53.04 ± 1.64 C	52.17 ± 1.44 C
3. Albumin (gm).**	1.36 ± 0.09 B	2.36 ± 0.09 A	2.48 ± 0.16 A	2.60 ± 0.17 A
4. Globulin (mg).**	2.30 ± 0.07 C	3.10 ± 0.18 B	3.48 ± 0.06 A	3.56 ± 0.07 A
5. Total protein (gm).**	3.66 ± 0.05 C	5.46 ± 0.24 B	5.96 ± 0.16 AB	6.16 ± 0.07 A

The averages of traits which have carried different letters horizontally indicate significant differences at 0.05 or 0.01.
NS = Non Significant. *: Significant differences at 0.05. **: high significant differences at 0.01.

This may be due to increase of feed intake as a result of nutritional payment of pregnant ewes in the last months of pregnancy, which leads to increase amount of microbial proteins produced by microorganisms (Thomas et al.,1994) and thus to increase absorption of amino acids by presence of folic acid and cobalt, and thus showed a significant increase in concentration of total protein in blood of pregnant ewes (Ali et al., 2005) that is one side and another side, it is possible that ewes had obtained folic acid and cobalt, which act as accompanying of enzymatic to contribute to synthesis of proteins by increasing effectiveness and activity of the liver, which is the source of the synthesis of blood proteins (Harper et al., 1994).The results obtained in table (4) indicate that there were a significant decrease in concentration of triglycerides in pregnant ewes which were treated with folic acid and cobalt in experimental treatments when compared to control of animals. This is due to significant improvement in amount of daily feed intake for ewes were treated with folic acid and cobalt to compared than control group, which was reflected to reduce acetone levels, which stimulated to energy representation process and improved of animal health due to reduced cytogenesis and ketogenesis (Pereira et al., 2013).So as was accompanied significant decline (P≤0.01) in concentration of cholesterol in second, third and fourth treatments than for control treatment. This was due to decrease in concentration of triglycerides in result of this study (table 4). The levels of triglyceride in esophageal blood serum were 68.26, 60.03, 53.04, 52.17 mg and cholesterol 159.14, 153.04, 142.00, 124.78 mg for the four treatments respectively. This results were consistent with results of Graulet et al. (2007) who found significant differences in concentration of triglycerides and cholesterol between four factors (control, vitamin B9, B12, B9 +B12).So as this results were consistent with Almahdawi (2018), who observed improvement significant on concentrations of blood proteins (total protein, albumin, globulin) of animal groups were treated with folic acid than for control group. Also this results did not agree with results of (Preynat et al., 2009) who did not found significant differences in concentrations of triglycerides and cholesterol in the blood serum of the milking heifers when using two diets, the first diet was called control diet (without folic acid) and the second contained 16 mg of folic acid in feeding of milking heifers. Another study, this results were consistent with results (Preynat et al., 2010) which found significant decrease in concentrations of total lipids and cholesterol in blood serum of dairy cows which treated with vitamin B9 or vitamin B9 + B12 compared to control group.

As for the lambs, the results indicated in table (5) that there were high significant differences (P≤0.01) between third and fourth treatments than for first treatments (control treatment) and second in concentrations of total protein and globulin. On the other hand, There were significant differences (P≤0.05)in concentration of albumins between the first treatment (control treatment) than for third treatment (cobalt) and fourth treatment (folic acid + cobalt). The concentrations of total protein were 3.84, 5.04, 5.70, 6.00 gm/dl and albumin concentration was 1.74, 2.22, 2.42, 2.54 gm/dl and globulin were 2.10, 2.82, 3.28, 3.46 gm/dl for four treatments respectively. As for cholesterol, the results showed high significant decrease (P≤0.01)in experimental treatments containing folic acid and cobalt and the mixture between of them when compared to the control treatment. The concentration of cholesterol was 152.10, 144.24, 139.80, and 128.58 mg respectively. The results of the statistical analysis appeared in table (5) that there were high significant differences between four treatments in concentration of triglycerides for blood serum of growing lambs during lactation period .The average of concentration of triglycerides was 62.86, 56.64, 49.24, and 39.15 mg for the four treatments, respectively.

Table 5. Effect of folic acid and cobalt on concentrations of cholesterol, triglyceride and blood proteins of Awassi lambs .

Traits	First treatment (control)	Second treatment (folic acid)	Third treatment (cobalt)	Fourth treatment (folic acid + cobalt)
1. Cholesterol (mg)**	152.10 ± 4.36 A	144.24 ± 1.49 AB	139.80 ± 1.27 B	128.58 ± 2.10 C
2. Triglycerides (mg).**	62.86 ± 0.81 A	56.64 ± 2.17 B	49.24 ± 2.73 C	39.15 ± 1.08 D
3. Albumin (gm).*	1.74 ± 0.30 B	2.22 ± 0.07 AB	2.42 ± 0.18 A	2.54 ± 0.17 A
4. Globulin (mg).**	2.10 ± 0.08 C	2.82 ± 0.19 B	3.28 ± 0.08 AB	3.46 ± 0.22 A
5. Total protein (gm).**	3.84 ± 0.28 C	5.04 ± 0.24 B	5.70 ± 0.11 A	6.00 ± 0.19 A

The averages of traits which have carried different letters horizontally indicate significant differences at 0.05 or 0.01.
NS = Non Significant. *: Significant differences at 0.05. **: high significant differences at 0.01.

Through the results were showed significant increase in concentrations of total protein and albumin in favor of the second, third and fourth treatments compared with the first treatment. This is due to the biological activity of the synergistic interaction between folic acid and cobalt which act as accompanied of enzymatic in synthesis of proteins due to the increased activity of liver, which is the source of synthesis of blood proteins (Harper et. al., 1994).

The results presented in table (5) for suckling lambs showed a significant decrease in mean concentrations of triglycerides and cholesterol in treated groups with folic acid and cobalt compared to the comparison group. This due to concentration of fat in the blood was always changes because the animal can ability to process of metabolize of fatty tissue and store in their body tissues as a result of the dissolution of fatty tissue to meet the energy needs of the animal (Saleh, 1985).On the other hand, this significant decrease in level of triglycerides in result of this study has leads to reduction of cholesterol level for experimental treatments than for control treatment. This results are consistent with results of Abou-Zeina et al.,(2008) who found significant increase in concentrations of blood proteins of Egyptian lambs group, which was consumed a diet that containing cobalt when compared with control group. As well as this result was consistent with results of Saadoun et al.,(2009) who observed high significant differences in concentrations of total protein and globulin and non-significant differences on rates of albumin concentration when he was using different levels of folic acid (0, 0.25, 0.50, 0.75 mg) by mouth dossage of Awassi lambs groups. As far as this results was agree with finding of Almahdawi et al., (2009) who found significant differences in averages of blood protein (total protein, albumin and globulin) when they were using different levels of folic acid (0, 0.60, 0.80 and 1.00 mg) in feeding of suckling lambs during lactation period.Also this results was consistent with results of Almahdawi (2018) who observed significant improvement in concentrations of blood protein (total protein, albumin, and globulin) which was injected with folic acid than for compared to the control group.

CONCLUSION

We conclude from of this study that use of folic acid and cobalt in treatment of some pathological injuries which caused by deficiency of one or both, such as anemia, weakness and atherosclerosis of ewes and their lambs. Also they found to share to formation of brain cells, nervous system, Red blood cells, DNA synthesis, building of fatty acids and energy production in the body. It was found that ejaculation of folic acid and cobalt was led to significant improvement in productive and reproductive performance of pregnant ewes and significant increase in the production of daily milk and its components such as milk protein, milk fat and lactose sugar when compared to control group. Also was found that the dosage of lambs with folic acid and cobalt led to increase of body weight and improved efficiency of food conversion and vitality which due to the improvement in concentration of blood proteins, which was accompanied by significant reduction in concentration of cholesterol and triglycerides of ewes and their lambs.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest

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