



## Effect of Spirulina Algae Powder and Folic Acid on Some Physiological Parameters During the Early Stages of Local ewe's Pregnancy in the Summer

Alan S.A. Salihi and Sarmad A.A. Alsaadi\*

*Animal Production Department, College of Agriculture, Kirkuk University, Kirkuk, Iraq.*

### Abstract

**F**OR A TOTAL of 83 days, from June 15, 2023, to September 6, 2023, this study was carried out in the sheep field of the Department of Animal Production, College of Agriculture, University of Kirkuk. The study examined the physiological effect of dosing pregnant ewes with an aqueous solution containing spirulina algae powder and folic acid alone or together during the early pregnancy stage. Sixteen local Awassi ewes' average weight of  $(40.67 \pm 0.470)$  kg and (1.5) years old were utilized in the experiment. Randomly divided into four treatments, there are four ewes for each treatment. The 1st treatment (control group) was given 50 ml of deionized water without any additives. The 2nd treatment was dosed with an aqueous solution containing 2.5 grammes of spirulina algae powder in 50 ml of deionized water (ewe/day). The 3rd treatment dosed folic acid twice a week at a dose of 20 mg/ewe. The 4th treatment dosed an aqueous solution of spirulina algae powder at a concentration of (2.5 grammes in 50 ml of deionized water/ewe/day) + folic acid at a dose of (20 mg/ewe) at two doses per week, which was a combination of the second and third treatments. Blood glucose rate, total antioxidant capacity (TAC), glutathione concentrations and Malondialdehyde levels improved in all treatment groups in comparing with control group at in the mid and end stages of pregnancy.

**Keywords:** Awassi, Spirulina, Early pregnancy, Physiological, Folic acid.

### Introduction

Sheep farming is one of the strengths of the Iraqi economy. Therefore, it was essential for the agricultural sector to grow to concentrate on its expansion. One of the most important agricultural species in Iraq is the sheep, which produces a lot of meat. As more people become aware of nutrition issues, the population's desire for meat is growing [1]. Rising temperatures will negatively impact the production of milk and meat, making it one of the biggest issues facing sheep breeders and consumers today [2, 3]. Oxidative stress and metabolic disturbances during pregnancy and lactation, which are frequent problems that endanger these animals' health and productivity, can put breeders in a tough financial situation. Antioxidants extracted from plant sources are therefore becoming more and more popular as a means of preventing oxidative stress [4, 5]. Therefore, both at the research and field application levels, efforts are being made to find new natural antioxidants that have a higher natural bioavailability in the environment than their synthetic counterparts. This initiative aims to reduce

the increasing global dependency on antibiotics in farm animal [6]. This is why algae, which are described as a broad class of organisms that range in size from tiny cells to massive seaweeds, have drawn attention [7]. Spirulina is an edible blue-green microalgae that is rich in essential amino acids, unsaturated fatty acids, proteins, vitamins, minerals, and visually appealing hues. Spirulina algae is easily absorbed and possesses therapeutic properties. Its qualities include antiviral, cleansing, anti-inflammatory, and regulating intestinal vitality [8]. Water-soluble vitamin B9, often known as folic acid, is essential for numerous physiological functions. Folic acid is essential for the synthesis of DNA, the creation of red blood cells, and cell division. It is especially important during periods of rapid growth and development, such as pregnancy and lactation [9].

### Materials and Methods

#### *Animals and duration of study*

The study utilized 16 mature ewes aged 1.5 years, weighing an average of  $40.67 \pm 0.470$  kg, obtained from the College of Agriculture at the University of

\*Corresponding author: Sarmad A.A. Alsaadi, E-mail: [dr.sarmadalsaadi@gmail.com](mailto:dr.sarmadalsaadi@gmail.com), Tel: 009647706112456 (Received 19/04/2024, accepted 18/06/2024)

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Kirkuk. The study was extended from June 15, 2023 to September 6, 2023 as an experimental duration. stayed for 14 days.

#### *Housing and feeding of study animals*

The animals shared lodging and food in barns that were partially open. For the purpose of feeding small ruminants, the National Research Council (NRC) for 2007 recommended that, in addition to the green fodder that was available in animal production fields, the ruminants be fed concentrated fodder twice a day, in the morning and the evening, in the form of dry coarse fodder (hay). Drinking water without restriction.

#### *Experiment design*

Sixteen Awassi ewes were randomly divided into four groups of four ewes each.

**Group I (T1):** Ewes given orally same quality of deionized water 50 ml.

**Group II (T2):** Given orally 2.5 g of spirulina algae powder dissolved in 50 ml deionized water/ewe/day.

**Group III (T3):** Given orally 20 mg/ewe dissolved in 50 ml deionized water/twice/week.

**Group IV (T4):** Given orally 2.5 g of spirulina algae powder dissolved in 50 ml deionized water/ewe/day + 20 mg/ewe dissolved in 50 ml deionized water/twice/week.

#### *Estrus synchronization and mating*

#### *Statistical analysis*

The complete randomization method (CRD). One-way full randomization was used to statistically assess the experiment's data. The significance of any variations in the coefficients was evaluated using both Duncan's multiple range test and Duncan's multiple test [10]. The following mathematical model was utilized to analyze the data using the already prepared statistical analysis tool SAS (2001):  $Y_{ij} = \mu + S_i + e_{ij}$

### **Results and Discussion**

The findings presented in Table (1) indicate that the concentration of glucose in the blood serum varies significantly ( $P \leq 0.05$ ). The blood glucose rate improved in the fourth treatment over all treatments in the mid-early stages of pregnancy, followed by the second treatment, the third treatment, and the control, in that order. Additionally, at the early pregnancy ended, and there was an improvement in the blood glucose level in the fourth treatment over all transactions, followed by the second, third, and first, in that order. The spirulina algae may have anything to do with the drop in blood serum glucose levels. The reason for this could be that the phycocyanin

The operation of unifying the estrus of ewes outside the reproductive season was performed using the method of vaginal sponges, and the operation was carried out according to the following program:

1. The ewes were given a dose of the prostaglandin hormone  $PGF_{2\alpha}$ .

2. The animals were immunized by giving them antibiotics plus an immunity booster.

3. Vaginal sponges with the addition of topical drugs were placed with the sponge to prevent the occurrence of vaginal infections, the survival time of the sponges was 14 days.

4. The sponges were taken out on the 14th day from the start of the experiment and Pregnant Mare Serum Gonadotropin hormone (PMSG) was administered in addition to the Equine chorionic gonadotropin hormone (eCG), which was attached to the sponges as part of the manufacturer's estrus consolidation program.

#### *Blood Sampling and Biochemical examination*

Blood samples were withdrawn after immobilization of animals for 12 hours from the Jugular Vein in the neck of the animal, where 8 ml was placed in special tubes for Serum separation, and then the blood serum was separated by a centrifuge 2900 cycles for 20 minutes, and the serum for each sample was distributed in Eppendorf tube capacity of 1 ml and tightly closed and kept at a temperature of  $-20\text{ }^{\circ}\text{C}$  until used for the required analyzes.

protein included in spirulina inhibits the enzymes that break down carbohydrates, such as alpha-amylase and alpha-glucosidase, preventing the intestines from absorbing as much glucose. By increasing hemoglobin levels, which in turn help to lower blood glucose concentrations, spirulina algae contribute to the reduction of glucose [11, 12]. The results showed that blood folate levels and insulin resistance in mammals as a whole had an inverse relationship, suggesting that increased folic acid levels are linked to enhanced insulin sensitivity [13]. The current results of experiment were consistent with the outcomes of [14, 15].

Table (2) shows that there were significant differences ( $P \leq 0.05$ ) in the level of total antioxidant capacity (TAC) between the experimental treatments. During the middle and end of the early pregnancy, the fourth treatment, surpasses all other treatments. The active ingredients in spirulina algae that function as antioxidants, such as pigments like phycocyanin C protein, flavonoids, phenols, beta-carotene, chlorophyll, tocopherol, vitamins E, and selenium, may be the cause of the rise in TAC levels. It prevents oxidative stress by giving up electrons or sacrificing itself [16]. One of the hydrophobic

peptides in water, Glycoprotein-13, is also present in spirulina algae powder. Numerous investigations have demonstrated that the hydrophobic peptide's ability to eliminate free radicals is due to the presence of unsaturated rings, like the imidazole ring [17]. As for folic acid, it contributes to higher levels of total antioxidants by promoting the production of more metallothionein protein, which has the direct ability to stifle free radicals and shield cells from radical damage [18].

According to Table (3) findings, there were significant differences ( $P \leq 0.05$ ) in the glutathione concentrations among the experimental treatments; in both the middle and late stages of early pregnancy, the fourth therapy (T4) performed better than all other treatments. Glutathione levels increased in the fourth treatment as a result of the antioxidant qualities of folic acid and spirulina, which also shield bodily tissues from free radical damage. This result was consistent with the results of [19, 20].

Table (4) presents significant differences ( $P \leq 0.05$ ) between the experimental treatments. The fourth treatment (T4) showed a significant improvement when Awassi ewes were dosed with a spirulina algae solution and folic acid, as compared to all other experimental treatments over the two experimental periods. The increase in total antioxidant capacity (TAC), which lowers or prevents lipid peroxidation, is the cause of this outcome and is displayed in the same table (2). Alternately, it might take on the function of the phycocyanin protein found in spirulina, which functions to suppress the primary producer of reactive oxygen species, the enzyme NADPH oxidase. It is one among the enzymes that uses NADPH as an electron donor to promote the transfer of electrons to  $O_2$  in order to produce superoxide or hydrogen peroxide [21]. Apart from its

significant function in controlling cellular redox processes and eliminating free radicals that lead to lipid peroxidation, beta-carotene in spirulina powder is also crucial [22]. Additionally, folic acid functions as a cofactor to activate glutathione peroxidase and helps to specifically boost the development of antioxidant enzymes like superoxide dismutase [23, 24].

### **Conclusions**

The treatments that were dosed with an aqueous solution of spirulina algae alone or in combination with each other showed a significant improvement, according to the data.

### *Acknowledgment*

The study was carried out at Kirkuk University's College of Agriculture in Iraq. Many gratitude to the employees in these disciplines and the administrative staff of the Animal Production Department for providing the facilities, requirements, and equipment.

### *Conflicts of interest*

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study.

### *Funding statement*

Self-funding.

### *Author's contribution*

All researchers participated in the research equally

### *Ethical approval*

Ethical approval was obtained from the University of Kirkuk, College of Agriculture, Department of Animal Production, which they prepared a ruminant field with its supplies for raising a flock of sheep.

**TABLE 1. The effect of dosing Awassi ewes with spirulina algae powder and folic acid on Glucose levels during early pregnancy (ng/ml)**

Treatment	Mid-early pregnancy	End of early pregnancy
T1 (Control)	74.97±0.73 <sup>a</sup>	83.17±0.98 <sup>a</sup>
T2 (Spirulina)	63.31±0.44 <sup>c</sup>	55.04±1.02 <sup>c</sup>
T3 (Folic acid)	66.89±0.89 <sup>b</sup>	71.84±0.40 <sup>b</sup>
T4 (Spirulina + folic acid)	57.38±0.80 <sup>d</sup>	49.08±0.80 <sup>d</sup>

\*The distinct letters inside the same column signify a significant difference between the treatments at the significance level ( $P \leq 0.05$ ),

\*Values were Mean ± standard error.

**TABLE 2. The effect of dosing Awassi ewes with spirulina algae powder and folic acid on TAC levels during early prancyncy (ng/ml)**

Treatment	Mid-early pregnancy	End of early pregnancy
T1 (Control)	3.34±0.09 <sup>c</sup>	3.44±0.32 <sup>c</sup>
T2 (Spirulina)	4.15±0.20 <sup>b</sup>	5.23±0.18 <sup>b</sup>
T3 (Folic acid)	3.49±0.11 <sup>c</sup>	3.69±0.12 <sup>c</sup>
T4 (Spirulina + folic acid)	4.80±0.18 <sup>a</sup>	6.02±0.09 <sup>a</sup>

\*The distinct letters inside the same column signify a significant difference between the treatments at the significance level ( $P \leq 0.05$ ),

\*Values were Mean ± standard error.

**TABLE 3. The effect of dosing Awassi ewes with spirulina algae powder and folic acid on Glutathione levels during early pregnancy (ng/ml)**

Treatment	Mid-early pregnancy	End of early pregnancy
T1 (Control)	1.18±0.04 <sup>c</sup>	1.15±0.03 <sup>d</sup>
T2 (Spirulina)	1.51±0.04 <sup>b</sup>	1.65±0.06 <sup>b</sup>
T3 (Folic acid)	1.27±0.01 <sup>c</sup>	1.31±0.03 <sup>c</sup>
T4 (Spirulina + folic acid)	1.78±0.06 <sup>a</sup>	1.87±0.03 <sup>a</sup>

\*The distinct letters inside the same column signify a significant difference between the treatments at the significance level ( $P \leq 0.05$ ),

\*Values were Mean ± standard error.

**TABLE 4. The effect of dosing Awassi ewes with spirulina algae powder and folic acid on Malondialdehyde levels d pregnancy (ng/ml)**

Treatment	Mid-early pregnancy	End of early pregnancy
T1 (Control)	2.71±0.07 <sup>a</sup>	3.63±0.10 <sup>a</sup>
T2 (Spirulina)	1.94±0.08 <sup>c</sup>	1.62±0.10 <sup>c</sup>
T3 (Folic acid)	2.40±0.06 <sup>b</sup>	3.15±0.11 <sup>b</sup>
T4 (Spirulina + folic acid)	1.49±0.09 <sup>d</sup>	1.13±0.02 <sup>d</sup>

\*The distinct letters inside the same column signify a significant difference between the treatments at the significance level ( $P \leq 0.05$ ),

\*Values were Mean ± standard error.

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## الأثر الفسيولوجي للنعاج المحلية المعالجة بمسحوق طحالب السبيرولينا مع أو بدون حمض الفوليك الى مرحلة الحمل المبكر في أشهر الصيف

نالان سردار عبد الكريم صالحى وسرمد عبد الرزاق عبود السعدي  
قسم الإنتاج الحيواني - كلية الزراعة - جامعة كركوك - كركوك - العراق.

### المستخلص

أجريت هذه الدراسة في حقل الأغنام التابع لقسم الإنتاج الحيواني، كلية الزراعة، جامعة كركوك. لمدة 83 يوماً من 2023/6/15 إلى 2023/9/6، تناولت الدراسة الأثار المترتبة على فسيولوجيا عند تجريع النعاج الحوامل بمحلول مائي يحتوي على مسحوق طحالب سبيرولينا وحمض الفوليك وحدهما أو مجتمعين خلال المرحلة الحمل المبكر. تم استخدام في التجربة ستة عشر نعجة عواسي محلية بمتوسط وزن  $40.67 \pm 0.470$  كغم وعمرها سنة ونصف. تم تقسيمها إلى أربع معاملات عشوائياً، بواقع أربع نعاج لكل معاملة. جُرعت النعاج المعاملة الأولى جرعة من الماء منزوع الأيونات دون أي إضافات وتم إعتبارها كمعاملة السيطرة، تم تجريع الحيوانات المعاملة الثانية بمحلول مائي يحتوي على 2.5 غرام من مسحوق طحالب سبيرولينا الذائبة في 50 ملليتر من الماء منزوع الأيونات لكل نعجة يومياً. وجرعت حيوانات المعاملة الثالثة بحمض الفوليك مرتين في الأسبوع بجرعة 20 ملغم/نعجة. وجرعت حيوانات المعاملة الرابعة بمحلول مائي من مسحوق طحالب السبيرولينا بتركيز (2.5 جرام مذاب في 50 مل ماء منزوع الأيونات / نعجة / يوم) + حمض الفوليك بجرعة (20 مجم / نعجة) بجرعتين أسبوعياً، والذي كان عبارة عن مزيج من المعاملتين الثانية والثالثة مقارنة مع مجموعة السيطرة، أشارت المعاملات التي تم تجريعها بمحلول مائي من مسحوق طحالب سبيرولينا مع وبدون حمض الفوليك إلى وجود فروق معنوية ( $P \leq 0.05$ ) في معاييرها الفسيولوجية.

**الكلمات الدالة:** سبيرولينا ، حمل المبكر ، فسيولوجيا ، حمض الفوليك ، عواسي.