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Hematological and Hormonal Effects of Spirulina Platensis Algae Powder and Zinc Sulfate on Iraqi Local Goats During Late Pregnancy

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Abstract: The motive of this study to investigate the impact of *Spirulina Platensis* algae powder and zinc sulphate on some haematological and hormonal parameters of female Iraqi- Local goats. 20 female goats were used, aged 2-3 years, average weight of 30.79 ± 0.47, divided randomly (n=5) into four groups. Group[1] did not receive any supplements (control group), Group[2] (Zn) received (50 mg zinc/kg diet), Group[3] (SP) received (5g spirulina/kg diet), Group[4] (Zn+Sp) received combined of zinc (50 mg/kg diet) and spirulina (5g/kg diet). The trial period was 180 days. The results indicated that (Sp) and (Zn+Sp) groups significantly ($P \leq 0.05$) superior in RBC, Hb, Hct, and significantly decreased ($P \leq 0.05$) the percentage of neutrophil cells in all treated groups compared to control. And a significant decrease ($P \leq 0.05$) of insulin level in the Sp and Zn+Sp treatments, with an improvement in insulin resistance in all treated animals compared to the control group, with a rise of T4 hormone in Zn and Zn+Sp groups and an increase of T3 in Goats that treated with Zn+Sp supplements. Generally, combination of spirulina platensis algae powder and zinc sulphate can be used to enhance physiological performance of field animals.

Keywords. Spirulina platensis, Zinc sulphate, Leptin, Insulin, Late pregnancy, Goat.

Introduction.

The late pregnancy stage is one of the critical periods that need follow-up and attention to the behavioral and physiological changes that occur in it [1]. Oxidative stress and metabolic disorders during late pregnancy are common problems in field animals, which impair their health and productivity, leading to economic losses for breeders [2]. Therefore, worldwide efforts are being made to discover new safe antioxidants from natural sources that have higher bioavailability and protective efficacy than synthetic ones in order to curb the increasing global use of antibiotics in animal production [3]. Researchers and scientists also focused on finding new economic solutions to prevent malnutrition that causes metabolic disorders in the body, and searching for functional nutritional supplements capable of fighting body-destroying diseases [4]. Hence the interest shifted to algae, which is defined as a heterogeneous assemblage of organisms whose size ranges from tiny, minute cells to giant seaweeds, and has been used as a healthy food source for centuries for both humans and animals due to its high nutritional value [5]. It has gained the utmost importance in order to address the prevailing energy, environment and food crisis in the world, and has become an important raw material for many applications in the world not only as nutritional supplements but also as medical therapeutics [6,7].

Spirulina is an edible blue-green microalgae with high nutritional value, as it is an important source of proteins, essential amino acids, unsaturated fatty acids, vitamins, minerals and important pigments. enteric [8]. Many studies have concluded the relationship of spirulina algae to improving health, growth, fertility, and increased production in animals [9]. It has not shown any chronic or acute toxicity or even adverse effects, making it safe to use as part of human diets or as a nutritional supplement to enhance the quality of ruminant diets [10].

The bioavailability of some free mineral elements in food is low and does not meet the needs of an animal [11]. And the trace minerals present in the feed are not sufficiently available to the animal due to their interaction with each other and their association with some chemical compounds such as phytate, oxalates and tannins, which prevents their absorption and deficiency in the body [12]. Due to frequent cultivation, grazing, deforestation, etc., the soil became deficient in most minerals, and then deficient in fodder crops [13]. Free radicals resulting from oxidative stress by interfering with the work of enzymes or acting as coenzymes and improving immune efficiency [14]. Its deficiency is not limited to the health and productivity of mothers only, but also affects the growth and vitality of newborns [15].

Zinc is one of the most studied minerals in the world in the twenty-first century because it practically participates in most of the biochemical and physiological processes that support the body of the organism [16]. It is considered one of the necessary minerals that requires the body to be supplied with it constantly, as it is the most deficient mineral element in food, and unlike other minerals, there is no reserve storage for zinc in the body [17]. It is an important component of a large and diverse number of metalloenzymes and acts as a catalyst for many enzymatic reactions [18]. It plays an important role in promoting growth and immunity, stimulating cell division and protein synthesis, maintaining the health and integrity of epithelial tissues, and is a stimulating factor for antioxidants. It has a role in milk production and stimulates the production and secretion of hormones [19].

Materials And Methods.

Study Animals and Experimental Design

The study took place in the ruminant fields of the College of Agriculture –University of Tikrit - Iraq. Used 20 female Iraqi local goats in the study, aged 2-3 years, with an average weight of 30.79 ± 0.47 Kg. Animals underwent a two-week acclimatization period, then were treated for 180 days, the treatments started 30 days before the oestrus synchronization, mating and continued through the pregnancy stages until near parturition. The animals were randomly divided into four experimental groups, with 5 goats per group. The 1st group (control) received the basal diet without any additions, 2nd group administered zinc sulphate capsules (Zn) 50 mg/kg diet daily, 3rd group (Sp) received spirulina platensis algae powder 5 g/kg diet added to the concentrated diet, 4th group (Zn+Sp) received combination of zinc sulphate (50 mg/kg diet) and spirulina platensis algae powder (5 g/kg diet). Spirulina Platensis algae powder was imported from PRC (Etuokeqi Kangsheng Spirulina co., Ltd). And Inorganic zinc (ZnSo4) was obtained from CDH company, India.

Reproductive stimulation

Oestrus in goats is synchronized out of the breeding season by vaginal sponge (60 mg Medroxy Progesterone Acetate) from HIPRA, Spanish company, was inserted into the vagina for 13 days. On the eleventh day post-insertion, 75 mg of PGF2α (Cloprostenol) was injected intramuscularly. Thirteenth days after sponge insertion, the sponge was removed and 500 IU pregnant mare serum gonadotropin (PMSG) was injected [20]. Then bucks were released for female mating, and the heat was detected after 24 to 72 hours of sponge removal.

Blood samples and laboratory analysis

Blood samples were collected 2 weeks before the expected date of parturition, using a 10 ml wine syringe directly from the jugular vein. The samples were centrifuged at 3000 rpm for 20 minutes to separate the blood serum and then stored at (–20 °C) until analysis. Hematology parameters, which included red blood cell count (RBC), haemoglobin concentration (Hb), Hematocrit (Hct), white blood cell count (WBC), lymphocytes, neutrophils, mean corpuscular volume (MCV), mean corpuscular Hemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were measured using the Automated Hematology Analyzer by (Erba Mannheim, Germany). leptin and prolactin hormones was measured using ELISA technology. While insulin, thyroxin (T4), triiodothyronine (T3), estrogen and progesterone by using a Cobas e 601 Device. Insulin resistance (HOMA-IR) was measured according to [21]. by following equation:

HOMA−IR = [glucose (mg/dL) × insulin (µU/ml)] / 405

Statistical Analysis

The data were analysed by statistical program (SAS) for one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test [22] to know the differences among the means at a significance level of 5.0% [23].

Results And Discussion.

Hematological parameters

The results in Table 1 show that there are significant differences in some CBC variables of female goats, as there was a significant superiority in RBC counts, Hb concentration, and Hct ($P \leq 0.05$) in Sp and

Zn + Sp treatments compared to the control group, while the increase of Zn treatment was not significant compared to the control group. The results also showed a significant decrease in the percentage of neutrophils in all of treatments compared to the control group, at same time, we did not find any significant differences between the treatments in WBC counts, immune lymphocytes and level of RBC indicators (MCV, MCH and MCHC). Because of the increase in the metabolic rate and oxygen demand during late pregnancy, it is good to increase the percentage of blood in the body within the normal range [24]. The significant increase in RBC counts, Hb concentration, and Hct volume in the treatments (Sp) and (Zn+Sp) may be due to the vital activity of the phycocyanin protein in spirulina algae powder, which is known for its beneficial effects on stem cells in the bone marrow, which enhanced the synthesis of red blood cells. [25]. Because of the good level of protein and iron in spirulina algae, the animals maintained iron stores (ferritin) during pregnancy, which reflected positively on the increase in hemoglobin concentration, as Layer et al. [26] mentioned that the protein affects blood formation by providing the amino acids necessary for the synthesis Porphyrin and globin protein thereby stimulating the synthesis of hemoglobin and the release of erythrocytes into the circulation. Also, the high levels of circulating iron in the blood, in conjunction with the need for excess oxygen during pregnancy, will facilitate the release of erythropoietin from the kidneys, which stimulate the production of red blood cells in the bone marrow, and thus increase the percentage of hematocrit [27]. Zinc may also contribute in another way to the formation of erythroid cells, by contributing to the expression of the structure of the GATA-1 protein, which is a transcription factor necessary for the differentiation and development of erythroid cells. Therefore, in case of zinc deficiency GATA-1 cannot be produced [28,29] These results agreed with [30,31]. As for the increase in neutrophil cells in the control group compared to other treatments, this increase in neutrophils may be due to the increase in reproductive hormone secretion that occurs under stressful conditions, and the negative energy balance that occurs in the animal during late pregnancy, stress can lead to an increase in the number of neutrophils At the expense of lymphocytes (evidence of stress) due to increased release of neutrophil reserves in the bone marrow, induced by glucocorticoids [32]. This result was in agreement with [33].

Table 1: Effect of zinc sulphate, spirulina algae powder and their combination on some hematological parameters of goats during late pregnancy.

Treatment / Parameters	Control	Zn	Sp	Zn+Sp
RBC ($\times 10^6$ /UL)	11.20 ± 0.58 b	11.94 ±0.78 ab	13.67 ± 0.52 a	13.25 ± 0.64 a
Hb (g/dL)	7.86 ± 0.51 b	8.61 ± 0.57 ab	9.89 ± 0.60 a	9.33 ± 0.78 ab
Hct (%)	24.12 ± 1.30 b	25.75 ± 1.70 ab	28.72 ± 1.24 a	27.93 ± 1.46 a
WBC ($\times 10^3$ /UL)	6.74 ± 0.70 a	8.17 ± 0.98 a	7.57 ± 0.77 a	8.35 ± 0.64 a
Lymphocytes (%)	53.49 ± 2.87 a	56.73 ± 2.97 a	56.17 ± 2.65 a	57.21 ± 2.74 a
Neutrophils (%)	37.95 ± 1.38 a	34.15 ± 1.43 b	34.79 ± 1.32 b	33.39 ± 1.30 b
MCV (fL)	21.82 ± 1.80 a	21.99 ± 1.74 a	21.19 ± 1.48 a	21.12 ± 1.26 a
MCH (pg)	7.13 ± 0.76 a	7.23 ± 0.30 a	7.28 ± 0.53 a	7.19 ± 0.57 a
MCHC (g/dL)	32.95 ± 1.49 a	33.68 ± 2.95 a	34.92 ± 2.45 a	33.92 ± 2.65 a

The values represent the mean ± standard error; Different letters within the same row are significantly differ at ($P \leq 0.05$).

Hormonal parameters

The results in Table (2) indicated that there were significant differences at a significant level ($P \leq 0.05$) in the level of insulin hormone and insulin resistance between the study groups, as found a significant decrease of Insulin hormone level in Sp and Zn+Sp treatments, while it did not differ significantly in the Zn treatment compared to control group. The results also showed marked improvement of insulin resistance in all treatments (second, third and fourth) compared to the control group. It may be due to the role of spirulina algae compounds, such as Ascorbic acid, Hexadecanoic acid, 9,12-Octadecadienoic acid, and gamma-linolenic acid, as these active compounds reduce the effect of free radicals from oxidizing the proteins of insulin hormone receptors, and their ability to activate these receptors and then increase the sensitivity of cells to insulin. [34]. It was also found that the components of spirulina algae, phycocyanin C protein, carotenoids, and vitamin E, have an important role in improving tissue resistance to the hormone insulin, by activating the hepatic mitochondrial signaling pathway (PGC-1α / Tfam / mtDNA) [35]. Or due to zinc modifying the transcription of the insulin receptor gene through zinc finger proteins, which are proteins that contain three zinc fingers that are necessary for binding, as the binding of these proteins is related to the possibility of expressing the gene coding for insulin receptors [36]. Our current results correspond with the findings of [37,38].

With regard to thyroid hormones, the results in Table 2 indicated that there were statistically significant differences ($P \leq 0.05$) between the experimental groups in the level of thyroid hormone and triiodothyronine, as it found a significant increase in T_4 hormone levels in the Zn and Zn+Sp groups compared to the control group, while the effect of SP treatment was negligible compared to the control group. As for the T_3 hormone, the fourth treatment (Zn+Sp) recorded a significant superiority compared to the control group. While the differences were not significant in the second and third treatment compared to the control group. Regarding the hormones leptin, estrogen, progesterone and prolactin, we did not notice any significant differences ($P \leq 0.05$) for these hormones between the experimental groups.

Table 2: Effect of zinc sulphate, spirulina algae powder and their combination on some hormonal parameters of goats during late pregnancy.

Treatments / Parameters	Control	Zn	Sp	Zn+Sp
Insulin (µIU/ml)	3.04 ± 0.41 a	2.19 ± 0.31 ab	1.84 ± 0.24 b	1.66 ± 0.13 b
HOMA-IR	0.53 ± 0.08 a	0.36 ± 0.05 b	0.28 ± 0.04 b	0.25 ± 0.02 b
Leptin (pg/mL)	1214.85 ± 69.78 a	1265.75 ± 87.57 a	1109.77 ± 61.18 a	1037.78 ± 50.29 a
T4 (nmol/L)	51.68 ± 3.72 b	57.33 ± 4.14 a	55.14 ± 3.91 ab	58.26 ± 3.33 a
T3 (nmol/L)	1.58 ± 0.11 b	1.82 ± 0.10 ab	1.74 ± 0.08 ab	1.91 ± 0.09 a
Estrogen (pg/mL)	117.83 ± 8.09 a	120.42 ± 8.25 a	121.93 ± 9.73 a	123.07 ± 7.94 a
Progesterone (ng/mL)	11.52 ± 1.11 a	12.15 ± 1.27 a	12.15 ± 1.27 a	13.06 ± 1.27 a
Prolactin (ng/mL)	4.03 ± 0.49 a	3.63 ± 0.33 a	3.84 ± 0.59 a	4.72 ± 0.61 a

The values represent the mean ± standard error; Different letters within the same row are significantly differ at ($P \leq 0.05$).

The significant increase of the T_4 and T_3 levels in treated groups may be attributed to the fact that spirulina algae powder is rich in minerals including iodine and selenium [39]. They are vital elements necessary for the functioning and health of the thyroid gland, as iodine is an essential element in the synthesis of thyroid hormones, and iodine deficiency in the thyroid gland leads to exposure to oxidative stress by increasing the thyroid-stimulating hormone (TSH), which increases its secretion to the thyroid gland and stimulates the formation of peroxide radicals. hydrogen H_2O_2 and then destroy and fibrosis the gland [40].

Zinc may also contribute to the protection of thyroid gland tissue, through its auxiliary role and component of antioxidant compounds, and its role in maintaining an appropriate level of metallothionein protein, which is a protective factor for functional chemical groups such as thiol from harmful free radicals [41]. This result was identical to the results of the researchers [42,43].

In conclusion.

In this study, we tried to inspect the effect of spirulina platensis algae powder and zinc sulphate alone or in combination on some physiological indicators during the late pregnancy period of Iraqi local goats. The obtained data show that use of spirulina and zinc gave positive results in some physiological variables (RBC, Hb, Hct and Neutrophils, insulin resistance and thyroid hormones), which gives the possibility of using spirulina platensis algae powder and zinc sulphate in order to control on physiological conditions of ruminants.

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