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Study on the Effect of Dietary Hemp Seed Powder Doses on Certain Reproductive Hormones in Iraqi Awassi Ewes

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Annotation: This study focused on examining how Russian hemp seeds affect specific reproductive hormones in Iraqi Awassi ewes. A total of twelve local Awassi ewes, ages between three and four years, It was selected and distributed into three experimental groups, each consisting of four ewes with similar body weights. Each ewe acted as an individual replicate. The groups were randomly assigned to each of the three treatment conditions: the first group (Control - T1) received no extra supplements; the second group (T2) was given a daily dose of 200 mg of powdered hemp seeds mixed with corn flour, which was administered in gelatin capsules; the third group (T3) received the same supplement as T2 but at a higher daily dosage of 300 mg. The treatment lasted for 45 days. No statistical differences (P>0.05) were detected in the levels of estrogen, LH, FSH, and prolactin in serum between groups. From the results obtained in current study, Level of hemp seed powder used (200 and 300 mg/ewe/day) are physiologically safe for Awassi ewes included in this study.

Keywords: Hemp seed powder, reproductive hormones, Awassi ewes.

Introduction

Hemp (Cannabis sativa L.) Annual herbaceous plant of the family Cannabaceae, is native to Central Asia. Hemp Hemp is an old drug with us for about 5 millenniums, and has been used as

food, medicine, drug and narcotic drug. Over time, hemp seeds have attracted a great deal of scientific interest owing to their excellent nutritional value and bioactive components, making them a precious dietary and medicinal food. Many previous studies have also documented the possibility to include hemp seeds or their oils into feed regimes for animals as a fat- and protein-rich alternative to increase product quality from animals, in particular milk and meat [1]. Even though cannabis is historically linked to psychoactive effects mainly due to delta-9-tetrahydrocannabinol (THC), it is not the case for the hemp seeds because this compound is at very low levels, thus they are suitable for humans and animals to consume [2]. From a nutritional point of view, hemp seeds are well balanced in terms of amino acid profile, a source of high quality complete protein, and unsaturated fatty acids, mainly ω-3 and ω-6, that occur in an ideal ratio of 1:3, which is proven to improve cardiovascular health and decrease the level of inflammation [3]. Moreover, these seeds are an important source of biologically active ingredients, among which the tocopherols (vitamin E), polyphenols, and phytosterols have been reported to display important antioxidant and anti-inflammatory activitiesTSS-1 [4]. Additionally, some phytochemical constituents of hemp seeds have been detected, such as hepatostatic phytoestrogens, which bind ERα and ERβ estrogen receptors, and can affect procreation in females and males. In fact, industrial hemp extracts have been reported as estrogenic, with some compounds binding estrogen receptors, and may therefore have influencing effects on reproductive physiology [5].

Materials and Methods

This study and the current experiment took place in the field of the College of Agriculture at the University of Kirkuk, Department of Animal Production, where 12 ewes of the Awassi breed were used, the ages of the ewes ranged from 3 to 4 years. The experiment was conducted in February and March of 2025 for a period of 45 consecutive days, The ewes were distributed into three groups, with 4 ewes in each group.The ewes were housed in semi-closed cages of equal size, with water and hay available ad libitum to each group. All ewes were raised under similar environmental conditions, and all experimental animals were given free access to a standard diet. The standard feed was tailored to the ewes' needs without any additives. Group 1 was the control group (T1) without any additives, Group 2 (T2) received a dose of 200 mg per ewe per day, and Group 3 (T3) received a dose of 300 mg per ewe per day. All ewes were given hemp seed powder daily, ground and mixed with corn flour in gelatin capsules. These substances were maintained throughout the experimental period (45 days). Blood samples were collected from Awassi ewes using a 5 ml syringe. The blood was added to glass tubes containing anticoagulants. Then, the serum was drawn and placed in 1 ml tubes and stored in a freezer until the required tests were performed for the experiment to measure prolactin, ovulation hormone, follicle-stimulating hormone and estrogen. After obtaining the laboratory analysis results, the results were analyzed according to the random design. To test the significance of differences between treatments, Duncan's multiple range test was used [6]. The mathematical model is as follows:$Yij = \mu + Ti + eij$ Where: Observation value j for treatment i = Yij, Overall mean for the trait under study = μ Effect of treatment Ti = I , Experimental error, which is assumed to be normally distributed and independent, with a mean equal to zero and equal variance σ^2 e = eij.

Results

Estrogen:

The results in Table (1) The results of the experiment did not show any significant discrepancy ($P>0.05$) in estrogen levels during the standard group (T1) and the two groups supplemented with Russian hemp seed powder (T2 and T3) during the experimental period of days (15, 30, and 45), as estrogen levels ranged between 0.36 and 0.50 ng/ml, indicating that adding doses of (200 and 300 mg/day) of hemp seed powder did not physiologically change estrogen levels compared to the standard group. Given the slight difference in the average decrease in the two groups supplemented with hemp seeds (T2 and T3) during the periods of 30 and 45 days, the size of the

effect was small and there were no significant differences in the statistical data.

Table (1). The effect of treatment with hemp seed powder on the concentration of the hormone estrogen (ng/ml) in Iraqi Awassi ewes. (mean ± standard error)

Periods Treatments	first period (1-15) day	second period (16-30) day	third period (31-45) day	total period (1-45) day
T1	0.50 ±0.02	0.49 ± 0.02 a	0.50 ± 0.02 a	0.496
T2	0.49 ±0.02	0.40 ± 0.03 a	0.38 ± 0.03 a	0.423
T3	0.50 ±0.02	0.39 ±0.03 a	0.36 ± 0.03 a	0.416

***Similar letters within the same column mean that there are no significant differences at the level (P>0.05)**

**** T1 control treatment without additives, T2 adding hemp seed powder at a concentration of (200 mg/day), T3 adding hemp seed powder at a concentration of (300 mg/day).**

LH Hormone

Table (2) no significant differences (P > 0.05) appeared between the two standards

the control group and both groups were supplemented with Russian hemp seed powder (T2 and T3) in LH levels during the experimental period. The average LH concentration on day 15 was very similar between groups T1 = 310, T2 = 309, and T3 = 310, and there were no significant differences. However, a slight and insignificant decrease was observed in the hemp seed supplemented groups T2 and T3 at 30 and 45 days of the experiment compared to the standard group, with the average on day 45 reaching 309, 294, and 290, respectively.

Table (2). The effect of treatment with hemp seed powder on the concentration of LH hormone (ng/ml) in Iraqi Awassi ewes. (mean ± standard error)

Periods Treatments	first period (1-15) day	second period (16-30) day	third period (31-45) day	total period (1-45) day
T1	310 ± 3.0	309 ± 2.5 a	309± 3.1 a	309.33
T2	309 ± 2.8	299 ± 3.2 a	294 ± 3.0 a	300.66
T3	310 ± 2.9	296 ± 3.1 a	290 ± 2.8 a	298.66

***Similar letters within the same column mean that there are no significant differences at the level (P>0.05)**

**** T1 control treatment without additives, T2 adding hemp seed powder at a concentration of (200 mg/day), T3 adding hemp seed powder at a concentration of (300 mg/day).**

FSH hormone

Table (3) It is clear from the table that there are no significant differences (P > 0.05) in FSH levels between the control group (T1) and the two groups treated with hemp seeds (T2 and T3). FSH levels at the beginning of the experiment were very similar between all groups, but there was a slight decrease in FSH levels in the two supplemented treatments at 30 and 45 days compared to the standard group.

Table (3). The effect of treatment with hemp seed powder on the concentration of FSH hormone (ng/ml) in Iraqi Awassi ewes. (mean ± standard error)

Periods Treatments	first period (1-15) day	second period (16-30) day	third period (31-45) day	total period (1-45)day
T1	500 ± 2.5	499 ± 3.0 a	499 ± 2.8 a	499.33
T2	498 ± 2.7	487 ± 3.1 a	481 ± 3.2 a	488.66
T3	497 ± 2.8	483 ± 2.9 a	479 ± 3.0 a	486.33

***Similar letters within the same column mean that there are no significant differences at the level (P>0.05)**

**** T1 control treatment without additives, T2 adding hemp seed powder at a concentration of (200 mg/day), T3 adding hemp seed powder at a concentration of (300 mg/day).**

Prolactin hormone

Table (4) shows that the addition of hemp seed powder at doses of 200 and 300 mg did not show any significant changes in prolactin levels compared to the control group during the experimental period. Levels ranged between 22.4 –27.5 ng/ml, with a very slight trend, which was not significant at the level of (P > 0.05), for an increase in both groups. The addition of hemp seed powder over the experimental period, particularly on day 45, was in the third and second treatments (T2 = 26.7 ng/ml) (T3 = 27.5 ng/ml) compared to the control group (T1 = 23 ng/ml).

Table (4). The effect of treatment with hemp seed powder on the concentration of the hormone prolactin (ng/ml) in Iraqi Awassi ewes. (mean ± standard error)

Periods Treatments	first period (1-15) day	second period (16-30) day	third period (31-45) day	total period (1-45)day
T1	22.4 ± 0.4	22.5 ± 0.4 a	22.3 ± 0.4 a	.2240
T2	22.9 ±0.4	24.3± 0.5 a	26.7 ± 0.5 a	.2463
T3	23.0 ± 0.4	25.2 ± 0.5 a	27.5 ± 0.5 a	.2523

***Similar letters within the same column mean that there are no significant differences at the level (P>0.05)**

**** T1 control treatment without additives, T2 adding hemp seed powder at a concentration of (200 mg/day), T3 adding hemp seed powder at a concentration of (300 mg/day).**

Discussion

The results of this study indicate that including hemp seed powder in the diet in doses 200 and 300 mg and they cannot affect anything (P>0.05) on the sex hormones, estrogen, luteinizing hormone (LH) and FSH as compared to the control group. Instead, these level of hormones stayed at or little lower in the control.

Hemp seed powder components (CBD and essential fatty acids, omega-3 and omega-6) as bioactives might be insufficient or unsuitable to ignite the rise in estrogen secretion and hence did not have an effect on the ovarian endocrine functions at the doses used This was agreed upon [7]. This result can in part be explained by the fact that hemp seed contains a number of cannabinoids - such as CBD and THC - whose secretion mediated by the testes, and also the hypothalamus and pituitary, have been suggested to suppress the release of various sex hormones

[8, 9]. Some reports also showed that Cannabis derivatives can influence the control of GnRH secretion in the mammalian model, leading to a decrease in LH and FSH levels in plasma [10].

In addition, the doses of 200 mg/day and 300 mg/day may be insufficient to induce a clear physiological response, or the hormonal response of Awassi ewes needs to take into account a delicate balance between diet compounds and how they interact with bioactive plant chemicals. The efficacy of the plant extract has been reported that depends on the species of animal, physiological state, duration of treatment, and processing method [11,12].On the other hand, despite the large con- tents in essential fatty acids as linoleic acid and alpha-linolenic acid in hemp seed, which may improve the reproductive potential, which was not seen to affect of the acid fatty in secre- tion of estragel while, As they indicated [13,14]

in regulation of FSH and LH, this findings showed that had a possibility of nothing influence of CANNs, It was suggested that cannabinoids have a strong inhibitory effects [15]. Prolactin level: A slight increase in serum prolactin level compared to the control treatment (T1). There was an increase in the levels of T2 and T3 compared to T1, but it was not significant ($P > 0.05$).

It is known that nutrition is a crucial determinant of the endocrine pattern of secretion in ruminants. The role of polypharmacy in increasing the risk of BB has been attributed to the presence of various antipsychotic medications in combination with other medications, co-morbid medical diseases including: diabetes diabestic complications, hypertension, chronic kidney disease, fatty liver, hypothyroidism and the presence of hyperpituitarism aside from the fact that the components in hemp seeds which are polyunsaturated fatty acids, phenolic compounds and flavonoids may have an indirect effect on Hypothalamic-pituitary axisIt suppresses the secretion of the hormone prolactin [16]. The mild rise in prolactin may be connected to the neuromodulator function of CB seed constituents, especially non-psychoactive cannabinoids, such as CBD that were reported to act on neuronal receptors related to the regulation of prolactin release [17]. Previous studies have demonstrated that the facilitation to prolactin release of CBD could be related to the modulation of serotonin and dopamine receptors [18], two of the main neurotransmitters regulating the release of this hormone. The reasons may be due to the presence of some minerals such as selenium or zinc or a combination of them in high concentrations in hemp seeds [19] [20], as well as the presence of fats at rates of 30 percent, which raises the level of some hormones. [21]. These findings are in line with previous research on the physiological and behavioral effects of plant-derived supplements From hemp seeds and trace elements in ruminants, highlighting the complexity of nutritional and environmental factors on reproductive and metabolic health [22] [23] [24], Despite daily supplementation of Russian hemp seed powder to Awassi sheep, significant differences were found in some behavioral traits of Awassi ewes. However, our results indicate a slight increase, which makes the safe use of Russian hemp seed and its inclusion in feed additives.

Conclusions and Recommendations

The present study found that 200 and 300 mg of Russian hemp seed powder per ewe for 45 days did not have any significant effects on estrogen, luteinizing hormone (LH), follicle-stimulating hormone (FSH), or prolactin levels. The obtained data suggest that such doses are physiologically suitable in non-preovulating Awassi ewes. According to findings of the present experiment, Russian hemp seed powder may be safely used as a source of antioxidants for supplementation in the diets of the ewes and without any adverse effects on risk or hormonal imbalance of reproduction.

Based on the results of our experiment, we recommend conducting experiments on higher doses of Russian or Indian hemp seed powder on different animals, both male and female, and studying reproductive hormonal changes, as Russian hemp seeds contain a small percentage of cannabidiol, and verifying the safety of use, in order to include it in ruminant feeds, as it contains a rich content of Omega 3, Omega 6, and essential minerals.

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