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Impact of *Curcuma longa* L. on Semen and Blood Parameters in Awassi Lambs

Khalid H Sultan

University of Mosul, College of Agriculture and Forestry, Animal Production Department, Iraq
***Corresponding author:** dr.khalid.h@uomosul.edu.iq

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ABSTRACT

The current study was conducted in a private field in Bibukht village, Mosul government, in order to evaluate the impact of *Curcuma longa* powder (CL) supplementation to the male Awassi ration on semen quality and some blood parameters. Twenty-four male Awassi lambs, with average body weight 47±1.23kg and 10-11 months old, were randomly assigned to two equal groups (12 lamb/group). The first group (control) was reared on a standard ration, whereas the second group was reared on a standard ration supplemented with CL (200mg/kg ration). The treatments lasted for 90 days. Semen and blood parameters were taken at 30, 60, and 90 days of treatment. Statistical analysis for the data revealed that CL supplementation caused a significant increase in ejaculate volume, concentration/ejaculate, concentration/mL, live sperm%, and individual motility compared with control, especially at the end of treatment (90 days), and a significant decrease in sperm abnormality% compared with control at $P<0.05$. On the other hand, CL treatment increased testosterone values and reduces estrogen values significantly as compared with control. In terms of blood parameters, CL treatment significantly improved most of the blood picture indices when compared to control, as well as serum total protein and globulin values when compared to control. In conclusion, CL addition to male Awassi lambs ration improves testosterone values and sexual activity as shown by the improvement of serum quality.

Key words: Awassi, *Curcuma longa*, Blood parameters, Semen, Sexual activity.

INTRODUCTION

One of the feeding methods used in recent years is enhanced ruminant adaptability and yield items, as phytogenic additions have so been common. Consequently, using natural products has a positive impact influence on people's health (Karásková et al. 2015). After the dangers of their use on animals, a number of chemical preparations such as growth stimulants, antibiotics, or hormones were used to enhance the growth and, as a result, they affect human health when consumed the product of these animals (Schwarz et al. 2001), as these chemical preparations promote bacterium resistance in humans ingesting those animals' goods (Hameed et al. 2020). Benkő et al. (2008) used some additives, such as probiotics, on some reproductive parameters and the hormonal status of female quail breeders, likewise, Abdul-Majeed et al. (2021) used nettle plant to improve some physiological and biochemical parameters in broiler chickens.

On the other hand, according to Molosse et al. (2019), adding curcumin to the diet of nursing lambs caused an increase in weight gain as well as anti-inflammatory and antioxidant effects, both of which have biological benefits for improving performance. Wencelová et al. (2015) recorded that medicinal herbs, as well as extracts containing essential oils, polyphenols, saponins, flavonoids and other secondary metabolites, can help to improve ruminal fermentation and metabolism.

As a result, feeding ruminants with herbal plants has become increasingly popular. However, the utilization of plants with nutraceutical qualities as synthetic promoters of growth replacements (García-Hernández et al. 2017) must be researched (FrAnKIČ et al. 2009; Khan et al. 2016). Furthermore, every feed plant additive should be examined due to the composition and secondary metabolites, varied action mechanisms, and the optimum dose for each physiological situation (Mendel et al. 2017). When using high-performance liquid chromatography, two additional curcuminoid components, demethoxycurcumin and bisdemethoxecurcumin, were found in very tiny quantities (1.62 and 0.98 %, respectively) when following the method of Coradini et al. (2014), which is discussed in detail by Jaguezeski et al. (2018). However, there is conflicting evidence about the effects of curcumin on male fertility indices. Several in vivo (Salahshoor et al. 2012) and in vitro (Bucak et al. 2010; Soleimanzadeh and Saberivand 2013) investigations strongly suggest that curcumin plays a role in the energy-promoting and protective actions on testicular tissue, spermatogenesis, and sperm cell oxidative balance. Curcumin has also been proven to counteract toxic

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effects on mal genital system caused by a number of physiological or environmental causes (Wei et al. 2009; El-Wakf et al. 2011; Khorsandi et al. 2013).

Turmeric supplementation of up to 2% dry matter concentrate has also benefited the growth performance and health of lambs (Odhaib et al. 2021). Curcumin has beneficial effects on the sexual glands, testis and ovary, which may be related to its antioxidative (Sahoo et al. 2008), anti-inflammatory (Farombi et al. 2007), anti-cancer (Cort et al. 2012), and anti-apoptotic properties (Aktas et al. 2012). The purpose of the current study is to determine and evaluate the efficiency of *Curcuma longa* on Awassi male lambs' semen quality and some blood parameters.

MATERIALS AND METHODS

Ethical Approval

All procedures were approved by the University of Mosul, College of Veterinary Medicine, Institutional Animal Care and Use Committee, ethical approval (UM.VET).

Experimental Animals

The research was conducted in a private field in the village at Baibukht, which is located northeast of Mosul, Iraq. In semi-open barns, twenty-eight Awassi male lambs 47±1.23kg body weight, 10–11 months old) were randomly assigned to two pens (n=12 each pen) and fed concentrate meals.

Experimental Design

Awassi lambs (24) were randomly divided into two groups (n=12). T1, the control group was fed a standard ration, whereas T2, the experimental group was fed a standard ration (Table 1) supplemented with 200mg/kg *Curcuma longa* powder (CL). CL was added to the diet on daily basis and mixed well, the experiment lasted 90 days. CL product was purchased from a local market (Alhadbaa, Mosul, Iraq).

Sampling of Blood

On days 30, 60, and 90 of the experiment, blood samples were obtained from lambs through jugular venipuncture into a vacuum tube containing (EDTA) as an anticoagulant as well as plan tubes. Blood samples were examined for red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) using an automatic hematology analyzer (Mytic18) from Orphee Swiss company. Blood samples in plan tubes were centrifuged at 3,000 for 15 minutes at 4°C. An automatic analyzer separated serum to assess biochemical blood profiles such as total protein (TP), albumin (ALB), and globulin (GLU). The kit prepared by Lake Forest, Ca 92630, Monobind, Inc. USA, was used to assess testosterone and estrogen concentrations.

Semen Collection

At 30, 60, and 90 days of the experiment, semen was collected from 24 Awassi lambs for all treatments by inserting an electrode of Electro ejaculator into the rectum and performing batches of electrical stimulation every 5-10 seconds to obtain an ejaculate, which was collected in a graduated tube and then located in a 37°C water bath for semen tests according to Fourie et al. (2004).

Table 1: Ingredients and nutritive composition of standard ration, determined on a dry matter*.

Ingredients of dry mater %		Chemical compound	%
Crushed barley	63	Dry matter	92.09
Soybean meal	11	Organic matter	94.52
Wheat bran	22	Crude protein	14.64
Wheat straw	3	Ether Extract	2.04
Food Salt	0.5	Crude fiber	8.15
$CaCo_3$	0.5	Ash	5.48
		ME (Kcal/kg/DM)	2534

*(Al-Khawaja 1987)

Semen Evaluation

Once a month, semen was collected. All collected & processing glassware were washed and sterilized with high-pressure steam, then dried and reheated at 35°C. The tube was immediately placed in a 37°C bath after ejaculation. The ejaculate volume (EV) was directly measured using the graduated collecting tube. Gallenhamp, No. 82TT8, Cat No. M/6-200-H HZ 60, England was used for microscopic investigation. Wave motion of fresh undiluted semen was assessed under a microscope ×10 with a 5 score to determine mass motility (MM). 5µ of diluted semen was placed on a warmed (37°C) slide to assess sperm individual motility. A drop of diluted (1:400) semen was deposited on a hemocytometer to count the number of spermatozoa. Evaluation of the survival of spermatozoa was evaluated using the eosin-nigrosine stain. On a clean glass slide, a small drop of semen and one drop of eosin-nigrosine stain were combined with a clean stick. A thin smear was created, dried with in air, and examined under a 400× magnification microscope. To assess the percentages of Live sperms (LS) and abnormality (AB), at least 200 spermatozoa were analyzed from each smear. pH-indicator paper was used to measure the pH of the semen.

Statistical Analysis

The data was analyzed using the Statistical Software Statics program (SAS, 2003), according to the Complete Randomized Design's two-way analysis of variance (CRD) utilizing (GLM) procedure. According to Steel and Torrie (1984), the differences between the means were determined using Duncan's multiple range test at the probability level ($P \leq 0.05$).

RESULTS

Effect of CL on Semen Parameters

Table 2 shows that after 30, 60 and 90 days of treatment, the CL caused a significant ($P<0.05$) increase in EV (1.11,1.18 and 1.26ml respectively) compared to control group (0.83, 1.05 and 1.12ml respectively), the superior increase was at 90 day of treatment. Also, a comparison between the control group with CL group exhibited a significant increase in CE at 60 and 90 day of treatment (1.99 and 2.37×10^9 respectively) compared to control group (1.67 and 1.78×10^9 respectively). A comparison between the CL group with control group exhibited a significant increase in CM at 90 day of treatment,

Table 2: Influence of *Curcuma longa* on semen parameters of Awassi males

Parameters	Treatments	Days		
		30	60	90
Ejaculate volume (ml)	T_1	0.83±0.02d	1.05±0.06c	1.12±0.03bc
	T_2	1.11±0.05bc	1.18±0.01ab	1.26±0.02a
Concentration	T_1	1.55±0.02d	1.67±0.04dc	1.78±0.04c
/ejaculate $\times 10^9$	T_2	1.58±0.04d	1.99±0.08b	2.37±0.01a
Concentration/ml	T_1	1.87±0.05a	1.62±0.09b	1.61±0.07b
$\times 10^9$	T_2	1.44±0.08b	1.61±0.09b	1.87±0.04a
Live sperms%	T_1	55.50±0.86d	58.62±0.96cd	61.00±0.70bc
	T_2	56.12±0.58d	64.12±2.27b	70.37±1.63a
Abnormality%	T_1	1.65±0.02a	1.57±0.02ab	1.57±0.03ab
	T_2	1.56±0.02ab	1.52±0.02b	1.20±0.04c
Mass motility	T_1	2.46±0.08c	2.87±0.04b	3.19±0.06a
	T_2	2.53±0.01c	2.90±0.04b	3.22±0.09a
Individual motility%	T_1	60.50±1.63cd	58.62±1.47d	61.87±1.98bcd
	T_2	63.25±0.83cb	65.50±0.48b	70.62±0.59a
pH	T_1	7.07±0.01a	7.10±0.02a	7.14±0.01a
	T_2	7.05±0.05a	7.04±0.01a	7.18±0.10a

T1 – Control, T2 – CL 200 mg/kg ration, $^{a.b.c}$ means different alphabets in a row differ significantly (P<0.05) for each parameter. EV-Ejaculate volume/ml, CE – Concentration /ejaculate, CM - Concentration /ml, LS - Live sperms%, AB - Abnormality%, MM - Mass motility/5.

Table 3: Influence of *Curcuma longa* on blood picture of Awassi males

Parameters	Treatments	Days		
		30	60	90
WBCs ($\times 10^3$µL)	T_1	7.91±0.11c	8.01±0.09c	8.04±0.11c
	T_2	8.15±0.06c	8.55±0.10b	9.23±0.16a
RBCs ($\times 10^6$/µL)	T_1	9.09±0.07cd	9.27±0.20cd	8.97±0.25d
	T_2	9.58±0.16c	10.29±0.13b	11.12±0.09a
Hb (gm/dL)	T_1	10.30±0.18c	10.32±0.16c	10.12±0.13c
	T_2	11.24±0.14b	11.46±0.15ab	11.84±0.03a
PCV (%)	T_1	28.81±0.29c	28.92±0.09c	29.93±0.19c
	T_2	29.98±0.06b	30.90±0.17a	31.20±0.10a
MCV (fL)	T_1	31.72±0.53b	31.30±0.80b	33.52±0.90a
	T_2	31.36±0.61b	30.05±0.28b	28.04±0.17c
MCH (Pg)	T_1	11.34±0.21ab	11.18±0.37ab	11.34±0.36ab
	T_2	11.75±0.22a	11.15±0.16ab	10.64±0.08b
MCHC (%)	T_1	35.76±0.60b	35.68±0.49b	33.83±0.52c
	T_2	37.49±0.47a	37.11±0.49ab	37.96±0.12a

T1 – Control, T2–CL 200 mg/kg ration, $^{a.b.c}$ means different alphabets in a row differ significantly (P<0.05) for each parameter.

Table 4: Influence of *Curcuma longa* on some serum blood biochemical traits of Awassi males

Parameters	Treatments	Days		
		30	60	90
Total protein (gm/dL)	T_1	6.42±0.04b	6.42±0.06b	6.40±0.03b
	T_2	6.74±0.04a	6.87±0.05a	6.88±0.12a
Albumin	T_1	2.84±0.03a	2.85±0.05a	2.92±0.09a
(gm/dL)	T_2	2.83±0.03a	2.96±0.07a	2.98±0.11a
Globulin	T_1	3.58±0.06b	3.57±0.09b	3.47±0.10b
(gm/dL)	T_2	3.91±0.04a	3.90±0.08a	3.89±0.08a

T1 – Control, T2–CL 200mg/kg ration, $^{a.b.c}$ means different alphabets in a row differ significantly (P<0.05) for each parameter.

1.87×10^9 and 1.61×10^9 respectively. LS increased significantly in CL group at 60 and 90 day of treatment (64.12 and 70.37% respectively) in comparison with control (58.62 and 61.00% respectively). AB improved significantly (P<0.05) in CL group (1.20%) compared to control (1.57%) at 90 day of treatment. The data obtained from Statistical analysis of MM and IM demonstrated a significant increase in MM in Cl group at 60 and 90 day of treatment (2.90 and 3.22, respectively) in comparison with control (2.87 and 3.19, respectively) and in IM (65.50 and 70.62%, respectively) compared to control (58.62 and 61.87%, respectively). No significant differences were recorded in pH between the treatments.

The results of hematological parameters of treated lambs at different days are shown in Table 3, a significant increase was observed (P<0.05) in WBC in blood samples of CL group at 60 and 90 day of treatment (8.55 and 9.23 $\times10^3$/µL) compared with control group (8.01 and 8.04 $\times10^3$/µL, respectively) and in RBC (10.29 and 11.12 $\times10^6$/µL) compared with control group (9.27 and 8.97 $\times10^6$/µL, respectively).

Effect of CL on Hematology Parameters

CL treatment caused a significant increase in Hb at 30, 60 and 90 day of treatment (11.24, 11.46 and 11.84gm/dL, respectively) in comparison with control group (10.30, 10.32 and 10.12 gm/dL, respectively), also CL causes a significant increase in PCV% at 30, 60 and 90 day of treatment (29.98, 30.90 and 31.20%, respectively) compared to control group (28.81, 28.92 and 29.93, respectively).

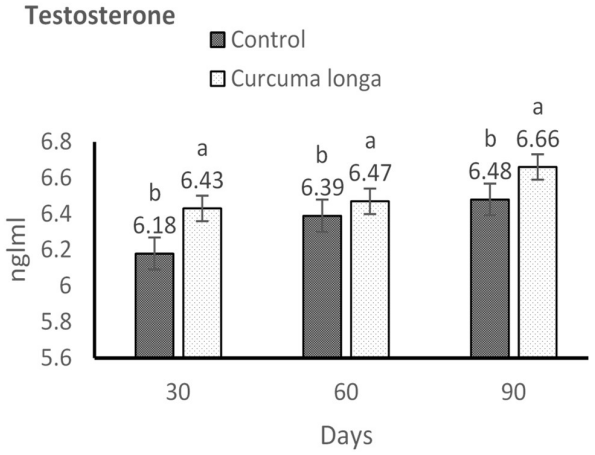

Fig. 1: Effect of *Curcuma longa* on Testosterone in Awassi males.

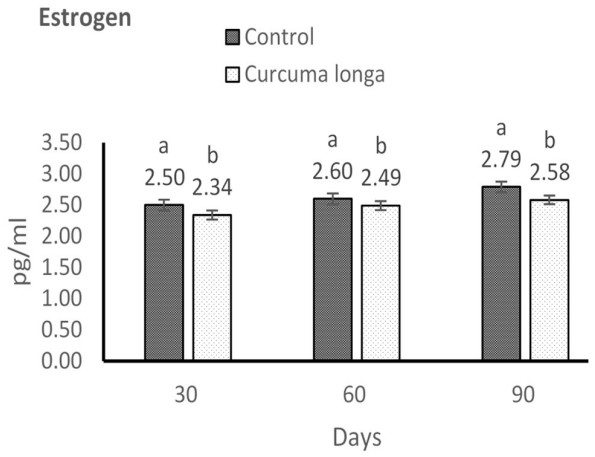

Fig. 2: Effect of *Curcuma longa* on Estrogen in Awassi males.

MCV decreased significantly at 90 day of treatment in CL group (28.04 fL) in comparison with control (33.52 micron^3), while MCHC increased significantly ($P<0.05$) in CL group at 30 and 90 day of treatment (37.49 and 37.96%, respectively) compared to control (35.76 and 33.83%, respectively).

Effect of CL on Serum Blood Biochemical Traits

Table 4 shows that CL supplementation (200mg/kg ration) caused a significant increase in the TP values (6.74, 6.87 and 6.88gm/dL) and GLU values (3.91, 3.90 and 3.89mg/dL) at all periods of treatment (33, 60 and 90 days) respectively compared with control (6.42, 6.42 and 6.40 gm/dL) for TP and 3.58, 3.57 and 3.47 gm/dL for GLU respectively at $P<0.05$. On the other hand, CL treatment had no effects on ALB along the whole treatment periods.

In regard for testosterone and estrogen values, Fig. 1 revealed that CL enhanced significantly testosterone concentration at 30, 60 and 90 day of treatment (6.43, 6.47 and 6.66ng/mL, respectively) in comparison with control (6.18, 6.39 and 6.48ng/mL, respectively) at $P<0.05$, also CL reduced estrogen concentration (2.34, 2.49 and 2.58pg/mL, respectively) along the whole treatment periods (30, 60 and 90 days) compared with control (2.5, 2.60 and 2.79pg/mL, respectively).

DISCUSSION

Recently, medicinal plants have been utilized to lessen the impact of oxidative stress on spermatozoa, which reduces sperm's capacity to fertilize eggs. Several studies have also focused on enhancing sperm quality and minimizing the impact of oxidative stress by supplementing sperm with antioxidants (Abdelnour et al. 2020). Statically analyzed data obtained in the current study showed a significant improvement in all semen parameters of CL group (200mg/kg ration), which was consistent with the findings of Tvrdá et al. (2015), whom added 0, 1, 5, 10, 50 and 100µM/L of curcumin to bovine semen. Moreover, Abdelnour et al. (2020), conducted a study in twelve buck's rabbits, pooled semen was cryopreserved in tris-yolk fructose extender supplemented with CL at levels of 0.5, or 1.5µg/mL, respectively, and observed a significant increase in sperm progressive motility and viability compared with control group, also the percentages of dead sperms and abnormalities were reduced significantly. In a study on semen of five mature bulls, Arboud et al. (2020) revealed the effect of CL on semen traits in diluted semen enriched with CL 0, 100, 200 and 300µL/5mL, and observed a significant improvement in semen motility and abnormalities. The results of current study were in agreement with the results of Kazemizadeh et al. (2019), who recorded a significant improvement in different parameters including semen concentration, total sperm production and progressive motility were linearly improved by the increasing levels of curcumin supplementation in broiler breeder roosters. In their study on adult Baladi bucks, Ismail et al. (2020) claimed that the enhancement of advance motility and viability belong to the kind of phenolic and flavonoid components in CL.

According to the Petruska et al. (2014), CL contains a lipophenol in soluble form that scavenges free radicals, and inhibits the generation of ROS. The authors reported that CL has an anti-oxidative effect to decrease lipid oxidative in the cell membrane of sperm and through down-regulation of the H_2O_2 level, which leads to protect spermatozoa from being injured (Zhang et al. 2017). The improvement of semen parameters might be associated with the portability of CL to reduce the protein and lipid oxidations (Abdelnour et al. 2020).

These results are in agreement with those of Odhaib et al. (2021), who observed a substantial increase in several blood parameters in their investigation on sixteen male lambs fed rations containing 1, 1.5, and 2% curcumin. In the current study, CL raised WBC, RBC, Hb, PCV, MCV, and MCHC. RBC and Hb levels may have grown over the course of the trial due to changes in metabolic needs brought on by the lamb's increased body weight and requirement for high oxygen levels. Moreover, Noori and sultan (2020) did not reported any significant differences in GLU, ALB, GLU and cholesterol in their study on twenty-four lambs reared on ration supplemented with CL (200mg/kg ration). Al Nahari and Al Eisa (2016) conducted a study to investigate the effect of curcumin on testosterone concentration in rats. The increase of testosterone concentration belongs to curcuminoids ability to improve spermatogenic damage and induce antioxidant defense.

Conclusions

We can conclude that CL (200 mg/kg ration) induced improvements in semen and hematological traits, when added to ration, and also significantly increased testosterone concentration in Awassi lamb's serum blood.

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Author's Contribution

Khalid H Sultan designed the plan of study, supervised the experiment, revised the manuscript writing and carried out language editing and formatting the manuscript.

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