

Sexual activity and reproductive performance of Ossimi sheep fed biologically treated rice
straw by enzymes or effective microorganisms

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ABSTRACT

The current study was carried out to investigate the effect of treating rice straw bales by enzymes (ZAD) or by effective microorganisms (EM) on sexual activity and reproductive performance of Ossimi sheep. Trial I, twenty-four lambs (12 males and 12 females) were randomly divided into three groups, first group fed control ration [concentrate feed mixture (CFM) and rice straw (RS)], and second group fed CFM and RS treated with ZAD. The third group fed on CFM and RS treated with EM. Body weight, age, T₃, T₄ and testosterone were determined at puberty, quality and quantity of semen collected from ram lambs and mature rams were also assessed. The fertility test assayed with mature rams and ewes. Results showed that age, body weight (BW), total gain and average daily gain values at puberty of both ram and ewe lambs were increased (P<0.01) in treated groups compared to control. Both ram and ewe lambs fed ZAD or EM reached puberty faster and levels of testosterone and thyroid hormones (T₃ and T₄) developed earlier than those of the control lambs. Moreover, ram lambs and mature rams in ZAD or EM groups were better in sexual activity and produced higher semen quality (P<0.01) than those of the control group.

Trial II, one hundred and fifty adult Ossimi ewes and three adult Ossimi rams without significant differences in semen characteristics were used in this trial. Rams given the previous three treatments. Ewes divided into three groups; first group (90 ewes) wasn't treated pre-mating (control), they fed control ration; and divided into three equal subgroups: A (n =30) mated by ram1 which not treated, B (n =30) mated by ram2 which treated by ZAD and C (n =30) mated by ram3 which treated by EM. Second group (30 ewes) treated by ZAD pre-mating and the third group (30 ewes) treated by EM pre-mating, and these two (2nd & 3rd) groups were mated by ram1 which not treated. Results showed that reproductive performance (reproductive ability, estrus duration, fertility rate, pregnancy rate, gestation period, lambing rate, fecundity rate, still birth and twinning rate) of treated sheep fed ZAD or EM were higher than those fed control ration. It could be concluded that enzymatic or microbial treatment to rice straw can improve reproductive performance of sheep.

Keywords: ZAD, EM, reproduction, semen, ewe, ram.

INTRODUCTION

Sheep's reproductive physiology differs from other animals, and other ruminants. Vital processes can be experienced at the fetal age of sheep, therefore the sexual activity as well. Ewe lambs become capable of having a normal pregnancy at age of 6-9 months, thus, they can start the main characteristics of their life performance: "the reproduction". Sheep have particular environmental needs: nutrition, environmental temperature etc. that may affect the sexual activity, According to the cyclical

function of the ovary, environmental and physiological factors influence both male and female sexual behavior (**Katz and McDonald 1992**).

Reproductive performance of rams is a complex trait because of several physiological processes such as the development of reproductive system from birth to puberty, spermatogenesis, ejaculation and mating behavior, involves libido and copulation. For optimum semen quality, all these physiological processes should be coordinated. Sexual behavior can be influenced by many factors,

including season of the year, genetics, breed differences, hormonal influences, post-weaning management, temperature, and nutrition (Mickelsen *et al.*, 1982). Puberty is a result of increasing gonadotropic activity and ability of gonads to simultaneously assume steroidogenesis and gametogenesis (Hafez and Hafez, 2000). Ram and ewe lambs that achieve early to puberty have special sexual behavior and can improve flock fertility (Ibarra *et al.*, 2000). Serum testosterone and estradiol levels showed a high correlation with sexual performance of rams (Abdullah *et al.*, 2002) and ewes (Fourie *et al.*, 2002) during mating. A peak of estradiol is associated with estrus behavioral of ewe. Sex hormones such as testosterone and estradiol are modulated by the hypothalamo-pituitary axis (Fourie *et al.*, 2002).

Nutrient preservation in silages is a result of fermentation by lactobacilli or other lactic acid producing bacteria. Several studies reported an improvement in nutrients digestibility by using microbial inoculates (El-Shinnawy, 2003; Abido, 2005; El-Ashry *et al.*, 2008; Abedo *et al.*, 2013; Khalifa *et al.*, 2013). Alc *et al.* (2010) found that microbial inoculants had a positive effect on grass silage characteristics in terms of lower pH and higher lactic acid concentration as grass silage established very well. Nutrition is a factor-influencing onset of lamb's puberty and has an important effect on sexual maturity and breeding age, slaughter weight and system of production which are closely related to the feeding regime (Fernando *et al.*, 2011). Exogenous enzymes (ZAD) and effective microorganism (EM) had positive effect on the development of immune organs and blood metabolites profile (Gao *et al.*, 2007; Esatu *et al.*, 2012); feed intake, digestibility coefficients, nutritive value and nitrogen balance (Gomaa *et al.*, 2012; Woju, 2010; Abdel-Khalek *et al.*, 2012); live body weight, age at puberty of lambs, and fertility rate of sheep (Khalifa *et al.*, 2013); ruminal fermentation, N balance and nutrient digestibility, as well as milk yield of cows (Gado *et al.*, 2007; Gado *et al.*, 2009), live body weight gain and feed conversion ratio of sheep and goats (Gado and Salem, 2008;

Gado *et al.*, 2011; Esatu *et al.*, 2012). Feed efficiency and digestibility of fat, protein (Freitas *et al.*, 2011) and amino acids (Angel *et al.*, 2011). The aim of this study was to evaluate the impacts of enzymatic or microbial inoculation to rice straw on sexual and reproductive performance of Ossimi sheep.

MATERIALS AND METHODS

This study was carried out to investigate the effect of biological treatments (enzymes; ZAD or effective microorganisms; EM) of poor quality roughage (rice straw) on reproductive performance of sheep.

The study carried out at Sids Experimental Station belonging to Animal Production Research Institute (APRI), Agriculture Research Center, Ministry of Agriculture, located at Beni Suef governorate in middle Egypt.

Ensiling procedures:

Rice straw bales ensiled in two heaps where the first one mixed with bacterial enzymes (0.3% ZAD) that already a compound of enzymes separated from anaerobic bacteria of the rumen. It contains a mixture of cellulase, hemicellulase, protease and alpha amylase enzymes (Gomaa *et al.*, 2012). The second heap of straw mixed with effective microorganisms (0.3% EM), which consisted of a mix of aerobic and anaerobic microorganisms with three major groups: i.e. photosynthetic bacteria, lactobacillus bacteria and yeasts and/or fungi (Higa and Wididana, 2007). The microbial inoculation was prepared as follows. Heaps covered by double-layer of linoleum plastic and straw bales closely tighten coverage. Samples from the two heaps were taken after 60 days for analysis to examine of roughage quality.

Trial I:

After weaning, twenty-four Ossimi lambs (12 males and 12 females) three months old of similar birth type were selected to compare effect of enzymatic or microbial treatment of rice straw by ZAD or EM on sexual performance and production of male and female lambs. Lambs averaged 16.56 ± 0.176 and 16.67 ± 0.167 kg body weight, respectively. Both sex of lambs were randomly divided into three

groups, first group (R1) fed control ration [60% concentrate feed mixture (CFM) and 40% rice straw (RS)], the second group (R2) fed on 60% CFM and 40% RS treated with ZAD, while, the third group (R3) fed 60% CFM and 40% RS treated with EM). All R1, R2 and R3 diets offered at two times; 9 am and 4 pm daily. Composite of feedstuff samples analyzed

according to the methods of AOAC (1995). All lambs weighed biweekly and kept under the same environmental conditions in semi-open shaded yard during the experimental period. Fresh water and blocks of mineral salts were available all times of the experiment. The compositions of feed stuffs and different rations are presented in Table 1.

Table (1): Proximate chemical analysis of feed stuffs and different rations:

Item	DM	OM	CP	EE	CF	NFE	ASH
CFM	90.64	91.72	16.20	4.53	15.82	55.17	8.28
RS	91.13	85.55	3.41	1.20	38.33	42.61	14.45
ZAD	90.51	82.01	6.08	1.78	31.08	43.07	17.99
EM	88.15	83.65	7.78	1.57	33.35	40.95	16.35
R1 (CR)	90.84	89.25	11.08	3.20	24.82	50.15	10.75
R2 (ZAD)	90.59	87.84	12.15	3.43	21.92	50.33	12.16
R3 (EM)	89.64	88.49	12.83	3.35	22.83	49.48	11.51

CFM, concentrate feed mixture used in formulating the experimental rations contained 24 % Cotton seed meal; 40% Wheat bran; 30% Yellow Corn 1.5% Lime stone; 1 % Sodium chloride, 0.5% vitamins and mineral mixture and 3% Molasses; RS, rice straw; ZAD, rice straw treated with enzymes; EM, rice straw treated with effective microorganisms; R1, CFM+RS; R2, CFM+RSZAD; R3, CFM+RSEM.

Puberty of ewe-lambs

Puberty recorded when female lamb exhibited its first estrus behavior. At 150 days of age, teaser rams introduced to ewe lambs to detect the onset of the first estrus. Teaser ram was introduced two times daily with 12 h interval at 6 am and 6 pm for each group for 60 min. Ewe lambs being receptive for teaser and stood for mounting by the teaser were considered in estrus. The onset of the first estrus used as an indicator for the onset of puberty. Date and body weight at first estrus recorded for each ewe lamb and considered as an indicator for pubertal age.

Puberty of ram-lambs

Sexual behavior was observed for all male lambs post-weaning until attainment of puberty (first collected ejaculate with motile sperm). Libido of each ram-lamb was measured within 25 min using female in estrus, considering the following criteria: first mounting without erection (stage I), mounting with 1st erection (stage II), and first collected ejaculate containing motile sperm (stage III).

Age, weight, scrotal circumference, and urethral process length were recorded at each stage of puberty. Scrotal circumference (SC)

evaluated using a flexible tape around the widest point of the testes (maximum circumference of the paired testes). The measurement of urethral process length was taken at the point of top and base dimensions by flexible tape.

Sexual performance and semen evaluation

Sexual performance tests were conducted during the morning hours between 8 to 10 am, two ewes in estrus were placed in two pens (one ewe/pen), a third similar pen separating the two testing pens was used for stood of the observer. Ewes kept unrestrained in the pens. Each ram was individually evaluated by exposing to the two ewes for 20 min on each test day. Two rams tested simultaneously while the remaining rams were kept about 20 yard away with visual barriers between them and the test pens. Rams randomly selected for testing, whereby each pen used to test rams of all groups on each test day.

Observations recorded for each ram throughout the 20-min period. Collected observations were similar to those reported by Kridli and Al-Yacoub (2006). They included bouts of reaction time and latency period.

After puberty until maturity, Rams sexually stimulated by allowing two false mounts especially before the first ejaculation.

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One false mount, followed by two minutes restraint, then a second false mount followed by another two minutes restraint ended by ejaculation (**Almquist and Hale, 1973**). Semen collected using an artificial vagina once a week between 8:00 and 10:00 am and continued for two months.

At each biweekly collection, two successive ejaculates were separately obtained for further processing. The temperature of inner liner-rubber sleeve-of the artificial vagina was adjusted to be 41-43°C at time of collection. Clean inner liner and graduated collecting tube were used for each collection. Lubrication of inner sleeve was performed using white sterile vaseline with sterile rod.

After collection, semen of each ejaculate transferred immediately to the laboratory where ejaculate volume measured directly in milliliters to the nearest 0.1 ml using a transparent graduated plastic tube.

For evaluation of percentage of sperm motility a drop of semen was examined under the low power, (x10) of a microscope using a hot stage adjusted at 37°C. Progressive motility was estimated in a percentage (%) score. Number of spermatozoa /ml of semen were determined using a haemocytometer. Total and motile sperm output / ejaculate and semen index were mathematically calculated.

Hormones assays

Blood samples were biweekly collected from each ram via jugular vein into heparinized tubes, centrifuged and plasma was stored at -20° C until analyzed for testosterone concentration by the method of **Jaffe and Behrman (1974)** who performed the assessment of total testosterone concentration using Coat-A-count 125I radioimmunoassay (RIA) kits purchased from Diagnostic products corporation, Los Angeles, California, 90045, USA. Triiodothyronine (T₃) and Thyroxin (T₄) were determined by radioimmunoassay procedures according to **Chopra et al., (1971), and Irvin and Standeven (1968)**, respectively by using kits purchased from Diagnostic products corporation, United states.

Trial II:

One hundred and fifty adult Ossimi ewes weighed 46.78± 0.68 kg and aged 40.3± 1.55 months had average parity 1.33±0.17. In addition, three adult Ossimi rams without significant differences in semen characteristics weighed 70 kg in average were used in this trial. Rams given the previous treatments.

Ewes divided into three groups. The first group (90 ewes) wasn't treated pre-mating (control), they fed basal ration (CFM+RS), and divided into three equal subgroups; A (n =30) mated by ram1 which not treated, B (n =30) mated by ram2 which treated by ZAD and C (n =30) mated by ram3 which treated by EM.

The second group (30 ewes) fed CFM plus RS treated by ZAD pre-mating, the third group (30 ewes) fed CFM plus RS treated by EM pre-mating. these groups mated by ram1 which not treated.

Measured parameters

1. Fertility rate (%) = (number of ewes pregnant / number of ewes mated) x 100
2. Pregnancy rate (%) = (number of ewes pregnant / ewes present to rams) x 100
3. Lambing rate (%) = (number of ewes lambed / number of ewes mated) x 100
4. Litter size (prolificacy) = (number of lambs / number of ewes lambed)
5. Fecundity rate (%) = (number of live births / number of ewes lambed) x 100
6. Still birth (%) = (Number of live births / Number of lambs born) x 100
7. Twinning rate (%) = (Number of twins / number of ewes lambed) x 100

Statistical analyses

Data statistically analyzed using the general linear model procedure (**SAS, 2002**). The differences among means tested using Duncan's Multiple-rang test (**Duncan, 1955**). The model used in statistical analysis was: $Y_{ij} = \mu + T_i + e_{ij}$

Where:

Y_{ij} = an observation

μ = overall means

T_i = effect of treatment (i = control, ZAD, EM)

e_{ij} = random error

RESULTS AND DISCUSSION

The present study aimed to study the effect of biological treatments of poor quality roughage (rice straw) on reproductive performance of Ossimi sheep.

Reproductive performance of ewe and ram lambs fed experimental rations post-weaning till puberty are illustrated in Tables (2) and (3). Results indicated that age and body weight (BW) at puberty; total gain and average daily gain until puberty of both sexes of lambs were significantly ($P < 0.01$) improved with treated lambs compared to the control one. Also, lambs pubertal weight and age in treated groups were significantly ($P < 0.01$) heavier and younger than those of untreated groups. The percentages of improvement in treated ewe lambs (ZAD or EM) were 21.38, 16.98% in age and 10.51, 5.24 % in body weights at puberty; 22.20, 15.53 % in total gain and 22.22, 15.56 % in average daily gain till puberty, (Table, 2), while, in ram lambs were 41.70, 37.02% in age and 27.37, 15.11 % in body weights at puberty; 51.74, 30.32 % in total gain and 51.79, 30.38% in daily gain till puberty, (Table, 3) in ZAD and EM groups, respectively compared to control group.

The improvement in puberty parameters of both sexes of lambs fed ZAD or EM may be related to improvement of energy intake. Similar trend has been observed by **Hosseini et al. (2008)** who found that energy is the major dietary element responsible for different utilizations of nutrients, thereby the productivity and body gain of an animal. The present results are in agreement with **Gomaa et al. (2012)** who reported that biological additive to Ossimi sheep's rations improved diets composition, nutrient digestion, and body weight. **Titi et al. (2008)** found that biological supplementation increased digestibility through enhancing growth performance, feed intake, or feed conversion ratio of fattened lambs and kids. The physiological processes related to puberty in some species had provided evidence for importance of dietary intake on sexual

maturity. **Khalifa et al. (2013)** confirmed that microbial treatment can preserve corn silage and can improve its chemical composition and ruminant value that was reflected on productive and reproductive performance of growing male and female lambs. Furthermore, microbial treatment provides a cheap energy and protein source for ruminant animals which reduce cost of feeding. **Chelikani et al. (2003)** reported that average daily gain and body weight together contributed 96% of the variation in attaining puberty. Available minerals may activate metabolic hormones signals that may play an important role in determination of onset of puberty. **Shirley et al. (2001)** found that heavier ewes tended to produce more growth hormone (GH) and attained puberty earlier than lighter ewes. **Wells et al. (2003)** suggested that thyroxin (T_4) might be an indicator of the onset of puberty because T_4 rises gradually from low concentrations just before onset of the breeding season to peak concentrations just before the transition to an estrus.

Thyroid hormones levels in Ossimi lambs as affected by ZAD or EM supplementation summarized in Tables (2 & 3). It noticed that thyroxin (6.18 & 6.03) significantly increased in treated lambs compared to the control group; while triiodothyronine (124.05 & 114.75) insignificantly increased in treated lambs compared to the control ones. T_3 and T_4 values proportionally correlated with the live body weight being lower in lighter and higher in heavier lambs (**Abd El-Hafeez et al., 2014**).

The differences in thyroid hormone concentrations among groups in the present study can partly attributed to differences in their respective metabolic activity due to differences in feeding level. Blood thyroid hormone levels are considered good indicators of the nutritional status of an animal and were correlated with feed intake in ruminant species (**Riis and Madsen, 1985**). There are reports suggested that energy deprivation decreased concentrations of both T_3 and T_4 (**Zhang et al., 2004; Todini, 2007**).

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Table (2): Puberty parameters of ewe lambs fed experimental rations.

Items	Experimental rations			Sig.
	CR	ZAD	EM	
Age at puberty (day)	265.00±7.64 ^a	208.33±6.01 ^b	220.00±10.41 ^b	**
Body weight at puberty (kg)	31.67±0.50 ^c	35.00±0.31 ^a	33.33±0.33 ^b	**
Total gain till puberty (kg)	15.00±0.58 ^b	18.33±0.33 ^a	17.33±0.67 ^a	**
Avg. daily gain till puberty (g)	100.00±3.85 ^b	122.22±2.22 ^a	115.56±4.44 ^a	**
T ₃ (ng/dl)	99.46±8.55	124.05±9.21	114.75±7.02	NS
T ₄ (µg/dl)	5.15±0.26 ^b	6.18±0.32 ^a	6.03±0.18 ^a	*

^{a-b} means in the same row followed by the same superscripts are not significantly different (P>0.05).

NS = Not significant (P > 0.05), * = (P < 0.05), ** = (P < 0.01).

Reproductive performance of ram lambs pre-pubertal, up to puberty and maturity showed in Table (3). Differences among the three groups were significantly higher (P < 0.05 & P < 0.01) for most reproductive parameters of ZAD and EM treated rams than control ones. Ages at puberty were 210, 164 and 183 days at 1st stage, 256, 190 and 228 days at 2nd stage, and 391, 228 and 246 days at 3rd stage, in CR, ZAD and EM groups, respectively. The obtained range of puberty stages are in agreement with those of **Khalifa et al., (2013)** and **El-Badawy (2003)** who reported similar pubertal age results. These results are in agreement with results of **El-Ashry et al. (2000)** who reported that Rahmani ram lambs reached puberty at age ranged between 36 and 40 weeks.

Testes circumference and plasma testosterone level were significantly improved (P < 0.001) in treated groups by ZAD and EM compared to the control group. In addition, urethral process length was significantly (P < 0.01) longer in treated group by ZAD or EM than that in untreated or control group. It is of interest to notice that the testes circumference and urethral process of rams throughout the experimental period appeared to be remarkably improved in the treated group than the control one, but they more improved in ZAD group.

In general, domestic ruminants require nutrients primarily for maintenance of their body metabolism, followed by growth, production and reproduction.

Testes circumference and testicular weight used in expecting the reproductive capacity of domestic male animals (**Sarder, 2005; Raji et al., 2008; Maurya et al., 2010**). Significant differences were evident between control group and treated groups (ZAD or EM) for testes circumference and urethral process in this study. The reason for this might due to the difference in energy supply through the feeding regimen applied in this study. Level of nutrition and daily gain could influence scrotal circumference (**Cameron et al., 1988**).

Gomaa et al., (2012) reported that treated RS with ZAD compound significantly (P < 0.05) improved the digestibility coefficients, chemical composition and fiber fraction of ration fed to Ossimi rams. They also reported that treating rations with ZAD compound increased TDN from 42.76% for the control group to 55.02% and 59.02% for the RS+ZAD group and RS+ZAD+ orange pulp group respectively, and increased digestibility of CP from 58.03% for control to 72.43% and 77.70% for RS+ZAD group and RS+ZAD+ orange pulp group respectively without any abnormal signs on blood and rumen parameters. **ELmoghazy et al., (2015)** reported that microbiological pretreatment of agricultural by-products with reference to rice straw was very important and significantly (P ≤ 0.001) maximize the nitrogen intake, urinary nitrogen and digested nitrogen which increase the use fullness of animal diets.

Table (3): Reproductive parameters of pubertal and mature rams fed experimental rations.

Items	Experimental rations			Sig.
	CR	ZAD	EM	
<u>1st stage: 1st mounting without erection:</u>				
Age (day)	210.67±2.33 ^a	164.67±8.37 ^b	183.00±4.41 ^b	**
Body weight (kg)	31.67±0.33 ^c	36.67±0.33 ^a	34.33±0.67 ^b	**
Testes circumference (cm)	15.33±0.33 ^b	19.33±0.33 ^a	16.33±0.33 ^b	**
Urethral process (cm)	2.50±0.29 ^a	3.00±0.15 ^a	2.67±0.17 ^a	NS
Testosterone level (ng/dl)	204.37±34.64	263.72±19.36	245.78±18.47	NS
T ₃ (ng/dl)	95.30±14.13	111.29±5.61	104.58±14.62	NS
T ₄ (µg/dl)	4.59±0.33 ^b	6.05±0.20 ^a	5.14±0.22 ^a	**
<u>2nd stage: 1st mounting with erection:</u>				
Age (day)	256.67±4.41 ^a	190.00±5.77 ^c	228.33±4.41 ^b	**
Body weight (kg)	34.33±0.33 ^c	40.30±0.30 ^a	36.67±0.33 ^b	**
Testes circumference (cm)	20.00±0.33 ^a	22.67±1.33 ^a	20.00±0.33 ^a	NS
Urethral process (cm)	2.96±0.05 ^b	3.33±0.04 ^a	3.28±0.03 ^a	**
Testosterone level (ng/dl)	262.16±34.13 ^b	317.24±5.99 ^a	294.53±32.16 ^a	*
T ₃ (ng/dl)	103.51±13.36	130.55±7.23	122.19±22.01	NS
T ₄ (µg/dl)	6.63±0.34 ^b	7.55±0.60 ^a	6.96±0.29 ^a	**
<u>3rd stage: 1st ejaculation, puberty:</u>				
Age (day)	391.67±4.41 ^a	228.33±4.41 ^c	246.67±3.33 ^b	**
Body weight (kg)	35.33±0.33 ^c	45.00±0.58 ^a	40.67±0.33 ^b	**
Total gain at puberty (kg)	18.67±0.67 ^c	28.33±0.67 ^a	24.33±0.33 ^b	**
Daily gain at puberty (g)	124.43±4.43 ^c	188.87±4.43 ^a	162.23±2.23 ^b	**
Testes circumference (cm)	22.00±0.58 ^b	27.00±0.58 ^a	25.67±0.53 ^b	*
Urethral process (cm)	3.00±0.29 ^b	4.33±0.17 ^a	4.00±0.20 ^a	*
Testosterone level (ng/dl)	325.96±10.66 ^b	380.41±18.52 ^a	340.91±10.25 ^{ab}	*
T ₃ (ng/dl)	119.06±18.82	159.66±14.39	134.97±24.44	NS
T ₄ (µg/dl)	6.50±0.29 ^b	7.69±0.38 ^a	7.02±0.19 ^a	*
<u>Mature rams</u>				
Body weight (kg)	69.33±0.82	70.76±0.72	69.74±0.88	NS
Testes circumference (cm)	27.81±0.19 ^b	31.48±0.50 ^a	30.94±0.07 ^a	**
Urethral process (cm)	3.50±0.29 ^c	5.00±0.20 ^a	4.33±0.17 ^b	**
Testosterone level (ng/dl)	423.03±10.92 ^b	548.84±17.68 ^a	514.20±17.06 ^a	**

^{a-c} means in the same row followed by the same superscripts are not significantly different (P>0.05).

NS = Not significant (P > 0.05), * = (P < 0.05), ** = (P < 0.01).

Highly significant positive correlation coefficients were found between body weight, testes circumference and testosterone levels in both Buffalo and Friesian bulls (**Abd El-Moty et al., 2001**). In addition **Sajjad et al. (2007)** reported that levels of blood serum testosterone correlate with scrotal circumference and semen volume.

In table (3), testosterone levels were low at early stages of sexual development in all

experimental groups and gradually increased with advance of age. The body weight and testicular circumference as affected by ageing might attributed to active testosterone secretion. Rams with bigger testes produce more testosterone levels. The increase in testosterone concentration at all stages such as first mounting without erection, first mounting with erection, and first ejaculation (puberty) were significantly (P < 0.05) better with ZAD and

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EM than control. The increase of testosterone levels might relate to improve in digestible energy. This finding is in agreement with that reported by **Scott et al. (2011)** who found that the mechanism underlying this sex specific effect of testosterone may due to sexual differentiation of the brain centers controlling energy expenditure.

Sexual activity parameters presented in Table (4). Sexual parameters were better in treated rams compared with the untreated ones. Reaction time and latency period were

significantly ($P < 0.01$) improved in treated rams by ZAD or EM compared to control ones. Pre-copulatory behavior correlated with ejaculation rate, which supports the hypothesis of **Price et al. (1992)** that frequency of pre-copulatory behavior in rams reflects their underlying sexual motivation. There are many factors affect sex drive and sexual performance. These factors include: season of the year, genetics, breed differences, hormonal influence, post-weaning management, temperature and nutrition (**Mickelsen et al., 1982**).

Table (4): Sexual activity of ram lambs at puberty as affected by experimental rations.

Items	Experimental rations			Sig.
	CR	ZAD	EM	
Reaction time (min)	6.88±0.01 ^b	5.25±0.01 ^a	5.22±0.01 ^a	**
Latency period (min)	11.69±0.3b ^b	9.18±0.24 ^a	9.49±0.31 ^a	**

^{a-c} means in the same row followed by the same superscript are not significantly different ($P > 0.05$).

**= ($P < 0.01$).

The shortest reaction time (5.25 and 5.22 min) for ejaculation recorded for ZAD and EM groups respectively while the longest was being in control group (6.88 min). The reaction time take for collection in treated groups and control significantly ($P < 0.01$) differed. Latency period also showed exactly similar trend as that of reaction time.

Ruminants require nutrients primarily for maintenance of their body metabolism, followed by growth, production and reproduction. In the present study, it showed that specific alterations in available dietary energy can have beneficial and long-lasting effects on animal physiology (**Martin et al., 2008**). Low, moderate and high levels of dietary energy intake can affect reproductive function in different ways. The maintenance of reproductive function requires a considerable amount of free available energy. Thus during times when food availability and energy supplies are low, it would make evolutionary sense to re-direct any available energy to maintaining brain function and cognition thereby affecting reproduction. Pre-copulatory behavior in rams reflects their underlying sexual motivation (**Price et al., 1992**).

Some physical semen characteristics for ram lambs, especially ejaculate volume,

progressive motility, sperm concentration, motile sperm, abnormal sperm and semen index at puberty and sexual maturity are shown in Table (5). The differences in average volume (ml) of semen among the three groups were highly significant ($P < 0.01$). The values were higher for treated groups compared with control group. Similar trend obtained for all other parameters (progressive motility, sperm concentration, motile sperm, abnormal sperm and semen index).

All physical semen characteristics at puberty and sexual maturity of treated rams were significantly higher ($P < 0.01$) compared to control rams. This improvement of semen characteristics could be related to the increase of body weight, testes circumference, in which they had higher values for treated rams than control ones (Table 3). In addition, testosterone had higher values in treated rams than control rams (Table 3). Testosterone plays the major role in development of reproductive organs and their functions (**Hafez, 1987**). **Yassen and Mohamed (1972)** found that there was a positive relationship between body weight and testicular size and their production of semen. During puberty, the androgenic effects resulting from increased testicular steroidogenesis are manifested by growth of the testes, external

genitalia and the male accessory reproductive glands (prostate, seminal vesicles and bulbourethral), and beginning of secretory activity. Furthermore, the secondary sexual

characteristics manifested during puberty can be divided into those that are a result of androgenic and anabolic effects (Kicman, 2008).

Table (5): Some physical semen characteristics of ram lambs at puberty and sexual maturity as affected by experimental rations.

Semen characteristics	Experimental rations			Sig.
	CR	ZAD	EM	
<i>Ram lambs at puberty</i>				
Seminal volume (ml)	0.27±0.03 ^b	0.60±0.06 ^a	0.53±0.33 ^a	**
Progressive motility (%)	26.67±3.33 ^b	53.33±3.33 ^a	45.00±2.89 ^a	**
Sperm concentration/ml (x10 ⁹)	1.22±0.06 ^b	1.57±0.09 ^a	1.46±0.04 ^a	**
Sperm output/ejac. (x10 ⁹)	0.33±0.05 ^b	0.96±0.15 ^a	0.78±0.04 ^a	**
Motile sperm/ml (x10 ⁹)	0.33±0.05 ^c	0.83±0.03 ^a	0.66±0.05 ^b	**
Motile sperm/ejac. (x10 ⁹)	0.09±0.02 ^c	0.50±0.06 ^a	0.35±0.04 ^b	**
Live sperm (%)	40.67±0.67 ^b	55.00±2.89 ^a	47.00±1.53 ^b	**
Abnormality (%)	21.44±0.61 ^b	15.24±0.43 ^a	16.51±0.63 ^a	**
Semen index	364.59±86.01 ^c	2779.00±437.50 ^a	1652.15±172.98 ^b	**
<i>Mature rams</i>				
Seminal volume (ml)	1.24±0.02 ^c	1.56±0.02 ^b	1.67±0.03 ^a	**
Progressive motility (%)	64.86±0.62 ^c	79.38±0.61 ^a	75.90±0.92 ^b	**
Sperm concentration/ml (x10 ⁹)	1.57±0.07 ^b	1.82±0.05 ^a	1.78±0.06 ^a	**
Sperm output/ejac. (x10 ⁹)	1.99±0.11 ^b	2.80±0.07 ^a	2.83±0.07 ^a	**
Motile sperm/ml (x10 ⁹)	1.19±0.06 ^b	1.68±0.05 ^a	1.54±0.06 ^a	**
Motile sperm/ejac. (x10 ⁹)	1.51±0.09 ^b	2.59±0.07 ^a	2.46±0.07 ^a	**
Live sperm (%)	70.08±0.64 ^c	84.61±0.70 ^a	81.26±1.00 ^b	**
Abnormality (%)	18.67±0.89 ^b	11.67±0.89 ^a	13.33±0.89 ^a	**
Semen index*	9349.56±588.62 ^b	19178.75±632.20 ^a	17738.75±601.45 ^a	**

^{a-c} means in the same row followed by the same superscript are not significantly different (P>0.05).

*Semen index = (semen volume × spermatozoa concentration/ml × live spermatozoa % × progressive motility %)

Improvement in testosterone levels (Table, 3) are good markers for semen quality and production. Testosterone hormone can directly affect the process of spermatogenesis, as normal spermatozoa are directly under the influence of sertoli cells which are responsible for sperm nourishment, division and caring as pointed out by Hafez (1993). Kishk (2008) reported significant correlation of testosterone with reaction time, semen volume, sperm motility and sperm concentration in rams. These results could explain based on effects of testosterone on testes functions especially on sertoli cells. The higher testosterone concentration in treated groups as compared to control group was similar to the findings of Malau-Aduli *et al.* (2003). They reported that additional nutritional supplementation aids

body weight gain and the peak of testosterone concentration in rams. The highest testosterone concentration recorded in ZAD group. This increase in testosterone concentration could be the reason for the better reproductive efficiency of ZAD group in the present study.

Data in Table (6) clearly indicate that rams or ewes fed ZAD or EM significantly (P<0.05) improved in their fertility and reproductive traits (reproductive ability, estrus duration, fertility rate, pregnancy rate, gestation period, lambing rate, fecundity rate, still birth and twinning rate) while insignificantly affected number of services per conception and prolificacy as compared to control groups. Reproductive performance of ruminants affected by four factors: genetic, physical environment, nutrition, and management. Nutritional factors

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are perhaps the most crucial in terms of their direct effects on reproduction. Moreover, nutritional factors, more than others, readily lend themselves to manipulations to ensure positive outcomes (Smith and Akinbamijo, 2000).

Table (6): Fertility parameters of rams or ewes fed experimental rations.

Items	Treated ram only		Treated ewe only		CR	Sig.
	ZAD	EM	ZAD	EM		
Total number of ewes	30	30	30	30	30	
Ewes present to rams	29	29	28	25	26	
Number of ewes mated	29	28	26	23	23	
Number of ewes pregnant	28	22	22	18	16	
Number of ewes lambing	28	22	22	17	16	
Number of lambs born	32	27	26	20	17	
Number of live births	30	25	24	17	13	
Number of twins	5	4	4	3	1	
Estrus duration (hour)	25.00±0.91 ^a	21.40±0.69 ^b	20.20±1.24 ^b	21.60±1.24 ^b	26.20±1.45 ^a	**
No of services / Concept.	1.10±0.06	1.11±0.06	1.19±0.08	1.26±0.09	1.30±0.10	NS
Fertility rate (%)	96.55±3.45 ^a	78.57±7.90 ^{ab}	84.62±7.22 ^{ab}	78.26±8.79 ^{ab}	69.57±9.81 ^b	*
Pregnancy rate (%)	96.55±3.45 ^a	75.86±8.08 ^{ab}	78.57±7.90 ^{ab}	72.00±9.17 ^b	61.54±9.73 ^b	*
Gestation period (day)	152.04±0.28 ^{ab}	151.59±0.39 ^a	153.14±0.65 ^b	153.59±0.67 ^b	153.50±0.50 ^b	*
Lambing rate (%)	96.55±3.45 ^a	78.57±7.90 ^{ab}	84.62±7.22 ^{ab}	73.91±9.36 ^{ab}	69.57±9.81 ^b	*
Litter size (prolificacy)	1.14±0.07	1.23±0.09	1.18±0.08	1.18±0.09	1.06±0.06	NS
Fecundity rate (%)	107.14±9.26 ^a	113.64±11.88 ^a	109.09±11.09 ^a	100.00±14.36 ^{ab}	81.25±12.30 ^b	*
Still birth (%)	93.75±4.35 ^a	92.59±5.14 ^a	92.31±5.33 ^a	85.00±5.19 ^{ab}	76.47±5.61 ^b	*
Twining rate (%)	17.86±6.52 ^a	18.18±7.37 ^a	18.18±7.22 ^a	17.65±7.18 ^a	6.25±4.34 ^b	*

^{a-b} means in the same row followed by the same superscript are not significantly different (P>0.05).

NS = Not significant (P > 0.05), * = (P < 0.05), ** = (P < 0.01).

Fertility rate (%) = (number of ewes pregnant / number of ewes mated) x 100

Pregnancy rate (%) = (number of ewes pregnant / ewes present to rams) x 100

Lambing rate (%) = (number of ewes lambing / number of ewes mated) x 100

Litter size (prolificacy) = (number of lambs / number of ewes lambing)

Fecundity rate (%) = (number of live births / number of ewes lambing) x 100

Still birth (%) = (Number of live births / Number of lambs born) x 100

Twining rate (%) = (Number of twins / number of ewes lambing) x 100

The present results agree with those of Al-Momani (2011) and Khalifa *et al.*, (2013) in sheep, Abdel-Khalek (2003) in Friesian cows, and Abdel-Latif (2005) in lactating buffaloes and buffalo heifers. These findings may partially due to improvement of digestibility of CP, CF, EE and NFE (Gado *et al.*, 2011). High feed intake suppressed circulating progesterone levels (McEvoy *et al.*, 1995). Steroids are necessary for the indication of estrous behavior in ewes (Hafez, 1993; Kinder *et al.*, 1995). The higher availability of

nutrients of ZAD and EM (Rodriguez *et al.*, 2002; Jurkovich, 2006) may increase the profitability of feeding which leads to higher conception rate and lambing rate. Higher lambing rate agrees with the finding of Islam *et al.*, (2007), Sabra and Hassan (2008) and Al-Momani (2011).

Flushing increases rate of ovulation, lambing rate and improve twinning rate (Islam *et al.*, 2007; Sabra and Hassan, 2008). The hepatic steroid metabolizing enzymes at flushing period thought to be associated with an increase in clearance rate of steroids. This is

associated with an increase in gonadotropins and consequently an increase in ovulation (NRC, 1985). Increase hepatic oxidative enzyme activity is proposed as a mechanism by which nutrient intake may influence ovulation rate (Smith, 1988). Flushing also increases the embryo survival (NRC, 1985). ZAD as enzymatic feed supplement improves the digestibility and nutritive value of feeds (Rodriguez *et al.*, 2002; Jurkovich, 2006; Gado *et al.*, 2009; El-Bordeny *et al.*, 2010) which increases the profit of the flushing treatment in ZAD group.

In conclusion, it could conclude that enzymatic or microbial treatment of rice straw could use to improve sexual activity pre and at puberty, thyroid hormones of ram and ewe lambs and testosterone levels of ram lambs. In addition, semen characteristics and fertility ability of adult rams and reproductive performance of adult ewes could improve by adding ZAD or EM to rice straw.

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النشاط الجنسي والأداء التناسلي للأغنام الأوسيمي المغذاه على قش الأرز المعامل بيولوجياً بالأنزيمات أو الكائنات الحية الدقيقة الفعالة

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أجريت هذه الدراسة لمعرفة أثر معالجة بالات قش الأرز بواسطة الأنزيمات (ZAD) أو الكائنات الحية الدقيقة الفعالة (EM) على النشاط الجنسي والأداء التناسلي للأغنام الأوسيمي. التجربة الأولى: استخدم عدد أربع وعشرين حمل (12 من الذكور و 12 من الإناث) قسمت عشوائياً إلى ثلاث مجموعات، المجموعة الأولى تغذت على عليقة الكنترول (العلف المركز وقش الأرز)، والمجموعة الثانية تغذت على عليقة العلف المركز وقش الأرز المعامل بال ZAD، والمجموعة الثالثة تغذت على عليقة العلف المركز وقش الأرز المعامل بال EM. تم تسجيل وزن الجسم و العمر وهرمونات التراى ايبودوثريونين، الثيروكسين و التستستيرون عند البلوغ، كما تم تجميع السائل المنوي من الحملان الذكور وكذلك الكباش الناضجة. أظهرت النتائج أن عمر ووزن الجسم وكذلك الزيادة الكلية فى الجسم ومتوسط الزيادة اليومية للجسم عند عمر البلوغ في كل من الجنسين من الحملان تأثرت بالمعاملتين مقارنة مع مجموعة الكنترول. وصلت كلا من الحملان الذكور والإناث المغذاه على عليقة ZAD و EM الى البلوغ أسرع وكذلك تركيز هرمونات التستستيرون والغدة الدرقية تطورت مبكراً مقارنة بالحملان المغذاه على عليقة الكنترول. علاوة على ذلك، كانت الحملان الذكور والكباش الناضجة المنتمية لمجموعتي ZAD أو EM أفضل في النشاط الجنسي وجودة السائل المنوي بشكل معنوي من تلك الموجودة في مجموعة الكنترول.

التجربة الثانية: واستخدمت مائة وخمسين نعجة وثلاثة كباش أوسيمي ناضجة دون وجود اختلافات كبيرة في خصائص السائل المنوي. وأعطيت الكباش المعاملات السابقة. ثم تم تقسيم النعاج إلى ثلاث مجموعات؛ المجموعة الأولى (90 نعجة) لم تعامل قبل التلقيح (كنترول)، وغذيت على العليقة الكنترول، وقسمت إلى ثلاث مجموعات فرعية متساوية: أ (ن=30) تم تلقيحها بالكباش الأول الذي لم يعامل، ب (ن=30) تم تلقيحها بالكباش الثاني الذي عومل بال ZAD، ج (ن=30) تم تلقيحها بالكباش الثالث الذي عومل بال EM. المجموعة الثانية (30 النعاج) عوملت بال ZAD قبل التلقيح، والمجموعة الثالثة (30 النعاج) عوملت بال EM قبل التلقيح، تم تلقيح هذه المجموعتان (الثانية والثالثة) بالكباش الأول الذي لم يعامل. وأظهرت النتائج أن الأداء التناسلي (القدرة الإنجابية، مدة دورة الشبق، معدل الخصوبة، معدل الحمل، فترة الحمل، معدل الولادات، معدل البقاء على قيد الحياة عند الولادة ونسبة التوأمية) للكباش والنعاج المغذاه على عليقة ZAD أو EM أعلى من تلك التي تغذت على العليقة الكنترول. يمكن استنتاج أن المعاملة الإنزيمية أو الميكروبية لقش الأرز كان لها تأثير إيجابي على جودة تخمر واستساغة المادة المائلة الفقيرة (قش الأرز) وتحسين الأداء التناسلي للأغنام.