EFFECT OF FEEDING WITH DRIED DISTILLERS GRAINS WITH SOLUBLES AND RICE POLISHINGS ON SCROTAL CIRCUMFERENCE AND SEMEN PARAMETERS OF RAM LAMBS[†]

[EFECTO DE LA ALIMENTACIÓN CON GRANOS SECOS DE DESTILERÍA CON SOLUBLES Y PULIDO DE ARROZ EN LA CIRCUNFERENCIA ESCROTAL Y PARÁMETROS DEL SEMEN DE BORREGOS JÓVENES]

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SUMMARY

The effects of level of dried distillers grains with solubles (DDGS) and rice polishings (RP) on the scrotal circumference and semen parameters were examined in 22 crossbred (Pelibuey x Dorper) ram lambs (average live weight 24.84 ± 2.07 kg, approx. 5 months of age). The lambs were randomly allotted to four treatments in a 2 x 2 factorial design. The two factors were dietary levels of RP (0 and 15%) and of DDGS (0 and 30%). The experiment lasted 52 days. The animals were weighed on days 0, 34 and 52, and semen variables were measured on days 34 and 52. Semen evaluation included sperm morphology, mass and progressive motility, and sperm concentration. On day 34, sperm concentration showed an interaction of both effects (P=0.01) and was greater in rams in group 4 (both RP and DDGS) than in those in groups T2 (RP alone) or T3 (DDGS alone). Sperm morphology (% normal cells) was higher (main treatment effect) in rams fed with 30% DDGS than in those not fed with DDGS (P<0.05). The other variables showed no treatment effect. On day 52, none of the ejaculate variables varied with treatments. In conclusion, the diets did not affect body weight of ram lambs, but did affect the percentage of normal spermatozoa at 34 d of the trial, although this effect was not observed after 52 d of the trial; thus, in the long run, these diets do not alter scrotal circumference or semen parameters of young Pelibuey x Dorper ram lambs.
Key words: Distillers grains; Rice polishings; Ram lambs; Semen characteristics.

RESUMEN

Se examinaron los efectos del nivel de granos secos de destilería con solubles (por siglas en Ingles, DDGS) y pulido de arroz (PA) en la circunferencia escrotal y parámetros del semen de veintidós borregos machos de la cruza Pelibuey x Dorper de peso vivo promedio 24.84 ± 2.07 kg y aproximadamente 5 meses de edad. Los animales fueron asignados en un diseño completamente al azar con arreglo factorial 2 x 2. Los factores fueron los niveles de PA (0 y 15%) y DDGS (0 y 30%) en las dietas. El experimento duró 52 días. Los animales se pesaron en el día 0, 34 y 52. Las variables de semen se midieron en los 34 y 52 días. La evaluación de semen incluyó morfología espermática, motilidad progresiva y total, y concentración de células espermáticas. En el día 34, la concentración espermática presentó interacción entre los factores (P=0.01) y fue mayor en los borregos del grupo 4 (15% PA + 30% DDGS) que en los animales de T2 (15% PA) o T3 (30% DDGS). La morfología (% células normales) fue más alta (efecto principal de tratamiento) en animales que recibieron 30% de DDGS que en animales sin DDGS en la dieta (P<0.05). Las otras variables no mostraron efecto de tratamiento. En el día 52, ninguna de las variables del eyaculado varió con los tratamientos. En conclusión, las dietas no tuvieron influencia en el peso corporal de los borregos, pero afectaron el porcentaje de espermatozoides normales a 34 días del estudio, pero este efecto no fue observado después de 52 d del estudio; por lo tanto, en periodos largos, estas dietas no alteran la circunferencia escrotal y parámetros del semen de los borregos jóvenes Pelibuey x Dorper.
Palabras clave: Granos de destilería; Pulido de arroz; Borregos jóvenes; características de semen.

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INTRODUCTION

Growth and economic viability of the sheep industry will not be profitable if not carried out in an orderly manner, organizing meat production, as some producers will focus on breeding stock, others on lamb production and others on feedlot lambs.

Dried distillers grains with solubles (DDGS) are an excellent source of energy and protein for sheep (Schauer *et al.,* 2008). Dried distillers grains with soluble are a byproduct of the ethanol industry, which are formed after the fermentation of cereal grains, mainly corn, by *Saccharomyces* yeast (Klopfenstein *et al.,* 2008). Large amounts of DDGS are obtained, providing a significant source of protein, amino acids, energy, phosphorus and other nutrients for feeding sheep and other domestic animals (Lumpkins *et al.,* 2005; Zhang *et al.,* 2010). They can be included in growing or finishing animals, lowering production costs compared with conventional ingredients such as sorghum grain and soybean meal (Schauer *et al.,* 2005). Therefore, the use of high levels of DDGS can be economically justifiable.

Rice is a grain of great economic and nutritional importance because it is widely cultivated; however, most production is destined for human consumption. For animal feeding, rice polishings (RP) are used; this feed is obtained when the surface of the endocarp is removed along with rice flour residues, and it constitutes approximately 20% of the amount of rice for human consumption. To make intensive sheep meat production more profitable, the use of available feed resources should be optimized, where RP can be part of the rations for sheep fattened in feedlots (Tabeidian and Sadeghi, 2009; Salinas-Chavira *et al.,* 2013).

Currently, both DDGS and RP are commonly used in diets for sheep but there is little information on the effect of these feeds on the reproductive efficiency of ram lambs. In a preliminary study, sheep fed with diets containing DDGS showed reduced sperm concentration (Van Emon *et al.,* 2013), the effect attributed to high sulfur levels in DDGS that reduced copper absorption of lambs and could negatively influence spermatogenesis. According to NRC (1996), RP shows high levels of crude fat (15%), NDF (26.1%), ash (10.4%) and sulfur (0.19%) which combined with the high levels of these nutrients in DDGS (10.7% crude fat, 46% NDF, 4.6% ash and 0.44% sulfur) may affect digestive and reproductive function of lambs. The information on this subject is scarce. Therefore, the aim of this study was to assess whether there is a negative effect of DDGS and RP on scrotal circumference and semen characteristics of ram lambs.

MATERIALS AND METHODS

This study was conducted at the College of Veterinary Medicine and Animal Science at the Autonomous University of Tamaulipas, Ciudad Victoria, Mexico. The study site is located at 23 °N and 97 °W, at an altitude of 340 meters. The average annual temperature is 24.3 °C, with an average annual rainfall of 926 mm.

The animal management protocol for this study was approved by the Bioethics and Animal Welfare Committee, FMVZ, UAT. The protocol followed the ARRIVE guidelines and the EU Directive 2010/63/EU for animal experiments.

Experimental treatments

The present study consisted of four diets, prepared using two levels of dried distillers grains with solubles (DDGS; 0 and 30%) and two levels of rice polishings (RP; 0 and 15%) in a 2 x 2 factorial design. Treatments were: T1 with 0% DDGS and 0% RP (control diet), T2 with 0% DDGS and 15% RP, T3 with 30% DDGS and 0% RP, and T4 with 30% DDGS and 15% RP (Table 1). In each case, the inclusion of DDGS and/or RP replaced a portion of sorghum grain, soybean meal and/or urea, to maintain similar levels of CP and ME. All diets were balanced to contain 14.8% CP and 2.8 Mcal/kg ME, considering the nutritional requirements of feedlot sheep indicated by NRC (2007).

Animals and management

The study was conducted in April and May 2015. Twenty-four non-castrated crossbred (Pelibuey × Dorper) ram lambs were housed indoors in individual pens that were 1.20 m long, 0.70 m wide and 1 m high, with individual feeding bunks. Lambs were allowed ad libitum access to feed and water. Feed was offered twice daily at 9:00 and 16:00 h. Offered feed and orts were weighed daily. The provided feed per day was approximately 110% of the prior day's consumption. Information of the feeding management of animals was not statically considered because the study was focused in the reproductive variables of animals. The ram lambs were randomly divided into four treatments with six repetitions (sheep). During the course of the study, two animals were eliminated from the study due to causes unrelated to the study, leaving at the end a total of six repetitions in T1 and T3, and five repetitions in T2 and T4. The adaptation period was 15 days and the experiment lasted 52 days.

At the beginning of the experiment the ram lambs weighed 24.84 ± 2.07 kg and were approximately 5 months old; they received vitamins A, D and E

intramuscularly (250,000 IU, 37,500 IU and 25 mg, respectively; Vigantol ADE®, Bayer, Mexico). An oral dose of 50 mg/kg of the anthelminthic Closantel (Closantel®, Panavet, Mexico) was given, with a second application 15 days later.

Reproductive variables

During the trial period, ram lambs were weighed and scrotal circumference was measured on days 0, 34 and 52; semen evaluation were performed on days 34 and 52.

Scrotal circumference (SC) was calculated with a flexible tape placed over the middle of the scrotum; the testicles were softly pushed toward the base of the scrotum and kept in that position during measurement. Daily scrotal growth for both sampling periods (34 and 52 d) was calculated.

Semen was collected and evaluated following the methods described by Evans and Maxwell (1990). One semen sample was obtained from each ram lamb on days 34 and 52 using an electroejaculator (Standard Precision, Denver, CO, USA).

Sperm motility was measured at two levels: mass motility and progressive motility. Mass motility was calculated by observing a fresh sperm preparation (at 100 ×) and scoring it on a 0-to-5 scale (0= no movement, 5= intense whirling motion). Progressive motility was evaluated by observing (at 400 ×) the number of sperm that moved more or less linearly in the microscopic field and estimated as a percentage of total sperm.

Sperm concentration was measured using a Neubauer chamber from semen diluted at 1:300. Sperm morphology was examined using semen smears stained with Rose Bengal; 100 cells were counted, distinguishing between morphologically normal and abnormal sperm.

Scrotal circumference and semen variables were compared between both sampling periods (days 34 and 52), to show the improvement in these variables as the rams matured.

Data analysis

Data were analyzed using a general linear model in a 2 × 2 factorial arrangement for unbalanced data, with 2 levels of dried distillers grains with solubles (0 or 30%) and 2 levels of rice polishings (0 or 15%). The model considered main effects and their interaction. Tukey's test was used to compare means when the interaction was significant. Student's t-test was used to compare semen variables between the two periods. The statistical models were evaluated using the GLM procedure of SAS. A significant effect was considered at $P \leq 0.05$.

Table 1. Percentage and nutrient composition of the experimental diets (% DM).

	0% DDGS		30% DDGS	
Ingredients	0% RP (T1)	15% RP (T2)	0% RP (T3)	15% RP (T4)
Sorghum stover	10	10	10	10
Sorghum grain	66.75	53.68	48.05	34
Soybean meal	14	11.3	1.85	0
Urea	0.5	0.57	0.04	0.05
Sugarcane molasses	5	5	5	5
Mineral supplement*	1.5	1.5	1.5	1.5
Salt	0.25	0.25	0.25	0.25
DDGS	0	0	30	30
Rice polishings	0	15	0	15
Tallow	2	2.7	3.31	4.2
Total	100	100	100	100
Nutrient composition on DM basis				
CP (%)	14.86	14.86	14.84	14.84
ME (Mcal/kg)	2.80	2.80	2.80	2.80

* Premix of macro and microminerals for feedlot lambs (Ovitec 302 F ®Tenusa Thrown; Monterrey, NL, México). Contains calcium carbonate, sodium bicarbonate, common salt, molasses, ammonium sulfate, ammonium chloride, magnesium oxide, ferric oxide (pigment), vitamin A acetate, Vitamin D3, vitamin E acetate, thiamine, antioxidant, ionophore (bovatec), aureomycin, artificial flavor, compounds of: manganese, zinc, iron, iodine, selenium and cobalt. DDGS=distillers grains with solubles; RP= Rice polishings.

RESULTS

Results for live body weight and semen characteristics after day 34 of the feeding trial are shown in Table 2. Sperm concentration showed an interaction (P=0.01) between factors, where concentration was greater (P < 0.05) in rams in group T4 (30%DDGS + 15% RP) than those in T2 (15% RP) or T3 (30% DDGS). The percentage of normal sperm was higher (main effect of treatment) in animals receiving DDGS than animals with no dietary DDGS (P = 0.04). There was no treatment or interaction effect on the other variables.

The corresponding results for day 52 are shown in Table 3. There was no treatment or interaction effect on live body weight or sperm characteristics (P > 0.05). Comparisons between variables recorded on days 34 and 52 are shown in Table 4. Values for live body weight, scrotal circumference, mass and progressive motility, and sperm concentration increased with age (P < 0.01). Daily scrotal growth or sperm morphology were not influenced by day of sampling.

DISCUSSION

In the present study ram lambs showed no difference among treatments and had an adequate nutrition regardless of group. The average live weight of ram lambs was 24.84, 36.27 and 40.11 kg at 1, 34 and 52 days of the feeding trial, respectively. Based on weight, the animals in the current study reached puberty, particularly at semen sampling periods (34 and 52 d). In other studies, puberty was reached at 21.4 kg in Dorper-cross rams (Villasmil-Ontiveros *et al.*, 2011), while in Pelibuey rams, it has been reached from 23.3 kg (Aguilar-Urquizo *et al.*, 2013) to 32.6 kg of live weight (Valencia-Méndez *et al.*, 2005). In the present study ram lambs at 52 days showed increased reproductive function, when the value for progressive motility approached the range of 70 to 90% described by Valdez (2013).

In agreement with the present study, Tufarelli et al. (2011) observed that scrotal circumference was not influenced by dietary treatments. In the current study, on average it was 29.09 and 31.86 cm at 34 and 52 days of feeding, respectively, which coincides with the 28.40 cm described for rams (Sarlós *et al.*, 2013). Aguilar-Urquizo *et al.* (2013) reported that scrotal circumference varied from 20.8 to 21.9 cm and live weight from 19.5 to 23.3 kg at the first normal ejaculate. They also reported that the onset of puberty in Pelibuey rams was not influenced by diet consumption of phytoestrogens in foliage from *M. Alba* or *H. rosa-sinensis.*

In the current study, scrotal growth was not influenced by dietary treatments; on average it was 0.13 and 0.15 (cm/d) at 34 and 52 days of feeding, respectively; these values are in agreement with Moreno-Cáñez *et al.* (2013), who reported daily testicular development of 0.15 cm/d in growing lambs. In other studies, nutrition improvements have enhanced reproductive characteristics in rams (Jiménez-Severiano *et al.*, 2010; Kheradmand *et al.* 2006).

The percentage of normal sperm on day 34 of the feeding trial was higher in animals receiving DDGS, while at day 52 of the feeding trial it was not influenced by dietary treatment. Overall average percentage of normal sperm in the present study was 63.91% and 66.27% at 34 and 52 days, respectively. These values exceed in 15% the minimum level required for semen used for artificial insemination (Hafez and Hafez, 2000). In part, results could be influenced by season because the study was conducted during the spring, when sheep show the highest percentages of sperm abnormalities (López *et al.*, 2011).

Sperm production in rams is influenced by health, age, conformation, nutrition, handling and environmental factors (Ghorbankhani et al., 2015). Testicular size, sperm production and quality are highest during the fall season (Hafez and Hafez, 2000; Zamiri *et al.*, 2010); at other times, semen quality may be lower, but values may be suitable for good fertility throughout the year (Zamiri *et al.*, 2010). In a tropical environment, Cárdenas-Gallegos *et al.* (2015) reported no influence of season on libido in Pelibuey, Blackbelly, Dorper and Katahdin rams.

Table 2.Growth and reproductive variables evaluated in ram lambs fed with different levels of distillers grains with solubles (DDGS) and rice polishings (RP; 34-d period).

	0% DDGS		30% DDGS		P value		
	0% RP (T1)	15% RP (T2)	0% RP (T3)	15% RP (T4)	RP	DDGS	Interac.
Initial weight (kg)	25.8 ± 0.8	25.8 ± 2.9	24.0 ± 1.2	23.3 ± 1.7			
Initial SC[1] (cm)	24.8 ± 1.7	24.7 ± 2.4	24.1 ± 2.5	24.6 ± 2.7			
Data at 34-d							
Weight (kg)	37.1 ± 2.8	36.4 ± 2.2	37.0 ± 2.3	34.3 ± 2.4	0.12	0.34	0.35
Scrotal circumference (cm)	29.0 ± 1.3	28.2 ± 1.3	29.8 ± 1.6	29.2 ± 1.3	0.24	0.14	0.90
Scrotal growth (cm/d)	0.12 ± 0.03	0.12 ± 0.04	0.15 ± 0.06	0.14 ± 0.06	0.68	0.32	0.75
Mass motility (0-5)	3.0 ± 1.8	1.6 ± 2.2	3.0 ± 1.7	4.0 ± 0.7	0.79	0.14	0.11
Progressive motility (%)	55.0 ± 24.3	40.0 ± 33.2	58.3 ± 17.2	70 ± 7.1	0.86	0.12	0.18
Sperm concentration (10^9/mL)	2.43 ± 0.99^{AB}	1.50 ± 1.36^{B}	1.73 ± 1.38^{B}	3.73 ± 1.06^{A}	0.31	0.24	0.01
Morphology (% normal cells)	60.5 ± 12.8	58.2 ± 6.2	73.2 ± 4.2	62.6 ± 10.8	0.12	0.04	0.31

[1]SC: scrotal circumference.
Within rows, values showing different superscripts differ ($P < 0.05$).

Table 3. Growth and reproductive variables evaluated in ram lambs fed with different levels of distillers grains with soluble (DDGS) and rice polishings (RP; 52-d period).

	0% DDGS		30% DDGS		P value		
	0% RP (T1)	15% RP (T2)	0% RP (T3)	15% RP (T4)	RP	DDGS	Interaction
Weight (kg)	40.8 ± 3.1	40.7 ± 2.2	40.6 ± 2.1	38.1 ± 2.6	0.24	0.24	0.30
Scrotal circumference (cm)	31.5 ± 2.6	31.0 ± 2.2	32.8 ± 1.3	32.0 ± 1.9	0.43	0.18	0.84
Scrotal growth (cm/d)	0.14 ± 0.12	0.16 ± 0.18	0.16 ± 0.10	0.16 ± 0.10	0.96	0.79	0.81
Mass motility (0-5)	4.3 ± 0.8	3.6 ± 1.5	4.2 ± 0.4	4.2 ± 0.8	0.40	0.66	0.36
Progressive motility (%)	68.3 ± 13.3	60 ± 29.2	70 ± 0.0	72 ± 8.4	0.64	0.36	0.46
Sperm concentration (10^9/mL)	5.28 ± 6.02	2.53 ± 1.83	6.00 ± 3.28	5.80 ± 2.33	0.38	0.27	0.45
Morphology (% normal cells)	67.2 ± 7.0	67.0 ± 6.0	66.2 ± 9.3	64.6 ± 3.8	0.78	0.58	0.82

Table 4.Comparison of the variables evaluated between both evaluation periods (34 vs. 52-d)

	34-d	52-d	P value
Weight (kg)	36.27 ± 2.52	40.11 ± 2.62	P < 0.01
Scrotal circumference (cm)	29.11 ± 1.41	31.86 ± 1.93	P < 0.01
Gross motility (0-5)	2.91 ± 1.77	4.09 ± 0.92	P < 0.01
Progressive motility (%)	55.91 ± 23.23	67.73 ± 15.41	P < 0.01
Sperm concentration (10^9/mL)	2.32 ± 141	4.97 ± 3.84	P < 0.01
Morphology (% normal cells)	63.9 ± 10.4	66.27 ± 6.6	P=0.19
Scrotal growth (cm/d)	0.13 ± 0.05	0.15 ± 0.12	P=0.20

Average sperm concentration for the four treatments was within the 2-6 × 10^9/mL range described by Evans and Maxwell (1990). As mentioned in the present study, lambs at 34 days of feeding had attained puberty, which allowed an increased sperm concentration by 52 days. Van Emon *et al.* (2013) studied Suffolk x western whiteface rams beginning at 40.4 kg (90 days old) and lasted 117 days; they reported that increasing dietary DDGS levels decreased sperm concentration. Despite some differences between the latter and the present study, semen evaluations ended at comparable ages. In the present study, animals receiving the diet with both DDGS and RP for 34 days showed a higher sperm concentration than those receiving either RP or DDGS alone. However, at 52 days none of the semen variables were influenced by dietary treatments. High dietary levels of sulfur in diets with DDGS can also alter the use of selenium and copper absorption (Richter *et al.*, 2012). This may explain the reduction in the concentration of sperm in rams consuming DDGS at 34 days; however this effect was absent at 52 days, indicating that, for the current study, sperm cell concentration was not affected by DDGS or rice polishing. It is unclear whether the difference at 34 days was due to diet (RP or DDGS) or to any of the other factors discussed above. Other studies in lambs have reported negative effects of feeds such as cotton seed and their by-products on the quality of the semen, especially on progressive motility and sperm vigor (Cunha *et al*., 2012; Paim *et al.*, 2016).

CONCLUSION

The use of 30% of dried distillers grains with solubles with or without rice polishings in diets for young Pelibuey × Dorper rams did not alter weight gain, scrotal circumference or quality of the semen after 52 days of feeding; therefore these feedstuffs may be safely supplied to these animals destined for breeding. Further studies should be undertaken for longer periods of feeding.

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