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Effects of dietary ferulic acid on reproductive function and metabolism of pre-pubertal hairbreed ewes during the anestrous season

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ABSTRACT

This study aimed to evaluate the effects of dietary supplementation of ferulic acid (FA) on reproductive tract weight (RTW), ovarian activity and serum concentrations of some metabolites and thyroid hormones of pre-pubertal hairbreed ewe lambs during the natural anestrous season. Twenty Dorper x Pelibuey crossbred ewe lambs (initial body weight = 28.5 ± 0.5 kg and age = 5.0 mo) were fed a basal diet and assigned to two treatments (n = 10) under randomized complete block design: FA supplementation to dosages of 0 (control) or 300 mg of FA/d animal^{-1}. Females were treated with FA during 34 d and then slaughtered to evaluate RTW and ovary. Blood samples were collected at days 1 and 34 to determine serum concentrations of metabolites (i.e. glucose, cholesterol, triglycerides, urea and total protein) and thyroid hormones. Final weight, dry matter intake, and serum concentrations of metabolites (except glucose) and thyroid hormones were unaltered by FA. Compared to control, FA-fed ewe lambs had lower ($P = 0.05$) serum glucose level but higher ($P \leq 0.05$) RTW, ovarian mass, number of larger follicles and corpus luteum (CL). While percentage of ewe lambs with small follicles and number of small follicles per female did not vary, percentage of ewe lambs with large follicles and CL increased ($P = 0.02$) with FA. In conclusion, FA supplementation improves the reproductive tract development and ovarian activity of pre-pubertal hairbreed ewe lambs during the natural anestrous season. An improvement in functionality of the glucose-insulin system could be related with this benefic effect of FA.

1. Introduction

Hair sheep are precocious breeds to reach puberty compared with wool sheep; while hair breeds can reach puberty between the ages of 6–8 months, wool breeds reach puberty between 8 and 12 months old [1]. Different factors can cause variation in the onset time of puberty in ewe lambs, despite the fact that they may have a suitable body weight to begin cycling (60% of the mature weight) [2]. Birth season is one of the factors that most influences onset of puberty, since ewe lambs born in winter or spring may reach puberty during the following reproductive season at an earlier age than those born in fall [3]. This longer onset time of ovulation in the autumn-born ewe lambs is attributed to a delay in the decrease of the responsiveness to oestradiol inhibition for tonic LH secretion, which in turn is induced by the photoperiod [4]. So ewe lambs should be exposed first to long days and then to short days to onset puberty. Given that hair breed sheep are less sensitive to photoperiod, some studies indicate that onset of puberty can be hastened in hair ewe lambs born in autumn [1,5]; however, it is necessary to develop some technology that promotes the occurrence of this event.

Ferulic acid (FA) is a natural phenolic compound present in plant cell wall components as covalent side chain [6]. This compound has been found in a wide variety of plants, including grasses and cereal grains used in sheep feeding [7,8]. Currently, FA is marketed in its free form and is a compound highly soluble in water, with low molecular weight (194.2 g/mol). Due to their chemical characteristics, free FA is rapidly absorbed in rumen and available in blood

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where it is transported by plasma protein toward peripheral tissues to exercise pharmacokinetic effects; then, this compound, in its original form or derivatives, is eliminated mostly in urine [9].

The pharmaceutical industry has shown great interest in FA because of their favorable effects on human health; FA is an ingredient of many drugs and functional foods, and also has nutraceutical effects [6,8,10]. Reproductively, FA has shown to be beneficial stimulating ovarian activity [11] and reproductive tract development [12] in female mouse, as well as in the maturation of pig oocytes and embryos *in vitro* [13].

Apparently, some reproductive processes in females are improved by the supply of phenolic compounds [14], including the FA [13], and the action mechanism is mainly associated to their powerful antioxidant effects [10,13,14]. However, using the rat as an animal model, the FA has been also effective in lowering blood levels of glucose, cholesterol and triglyceride by increasing insulin, modulating the expression of lipogenic and gluconeogenic genes in liver, and by enzymatic action [6,7,15]. Additionally, higher synthesis of estrogen in female mice [15] and growth hormone in heifers [16] were detected by FA effect. Given that all reproductive events are regulated by metabolic and endocrine signals, it is likely that other mechanisms, different from those of antioxidants, are acting to improve the reproduction processes of females treated with FA.

Notably, no studies were found in the literature reporting FA effects on reproductive aspects of hair or wool breed sheep. Based on all this background described above, we hypothesized that dietary FA stimulates the reproductive tract development and onset of puberty in pre-pubertal hair ewe lambs during the anestrous season as a result of its antioxidant effect and activation of metabolic mechanisms. Therefore, the objective was to evaluate the effects of dietary supplementation of FA on reproductive tract weight, ovarian activity and serum concentrations of some metabolites and thyroid hormones in pre-pubertal hairbreed ewe lambs during the natural anestrous season.

2. Materials and methods

2.1. Study site

The study was conducted during the natural anestrous season (April–May) at the Sheep Experimental Unit of the Instituto de Ciencias Agrícolas, Universidad Autónoma de Baja California, located in the Mexicali Valley, Baja California, at the northwestern Mexico (32° 8′ NL, 114° 6′ WL). The climate is warm desert (BWh), with maximum mean temperatures >40 °C in summer and scanty rain (85 mm annual) [17].

2.2. Animals, management and treatments

The management that received sheep females was according to approved Mexican Official Standards for the production, care, use of laboratory animals and their slaughter (NOM-62-ZOO-1999; NOM-033-ZOO-1995). Also, methods and managements applied to the animals were in strict accordance with accepted guidelines by the FASS [18].

A group of 34 Dorper × Pelibuey pre-pubertal ewe lambs born in autumn season (during November) was selected for this experiment. Sheep females were treated with vitamins (A-D-E; 1 mL/animal of Vigantol, Bayer Laboratory, Mexico) and against parasites (0.5 mL/animal of Ivermectina, Sanfer Laboratory, Mexico), as well as housed in individual pens and adapted to a basal experimental diet 22 d before the start of the experimental phase that lasted 34 d. On days 12 and 22 of the adaptation period, the lack of reproductive cyclicity was verified scanning by ultrasonography both ovaries in each ewe lamb (transducer 3.5/7.5 Mhz, Draminski ultrasound, Animal Profi model, Poland). Ewe lambs without presence of *corpus luteum* (CL) in both scans were selected to perform the experimental phase.

At the beginning of the experiment, ewe lambs were individually weighted (initial BW = 28.5 ± 0.5 kg and age = 5 mo) and assigned under a randomized complete block design to one of the following dietary treatments: 1) only fed the basal diet (control), and 2) fed the basal diet plus daily supplementation of 300 mg of FA per female (supplemented). In order to guarantee the total intake of the phenolic compound in supplemented ewe lambs, 111 g of FA were mixed with 10.9 kg of wheat meal, and 30 g/d of mixture was offered to each ewe lamb during the morning feeding. At the same time, ewe lambs from control group were fed 30 g/d of wheat meal. Daily dosage of FA was determined according to recommendation suggested in the patent entitled “ferulic acid as feed supplement in beef cattle to promote animal growth and improve the meat quality of the carcass and the meat” (publication number: US20130041036A1) [19]. That patent indicates that a daily dosage of 250 mg/kg of food maybe included in the diet for finishing cattle in fattening; so we estimated the average daily feed intake considering that this is equivalent to 4% of the initial BW (i.e. 1.14 kg/d and adjusted to 1.2 kg/d), and then we estimated the daily dosage of FA that should be included based in the estimated daily feed intake (300 mg/d). In this way, a daily dosage of 300 mg of FA per female was calculated to offer considering an average daily feed intake of 1.2 kg. In general, ewe lambs were fed twice daily at 700 and 1700 h in a 50:50 proportion. All animals had free access to water. Health status of each ewe lamb was verified daily by direct observation, and no female showed signs of illness.

The basal diet was formulated to meet fattening requirements [20] using the following ingredients: wheat meal (55.8%), alfalfa hay (28%), cottonseed (10%), cane molasses (4%), limestone (1%), calcium phosphate (1%) and grain salt (0.2%). Food samples were collected and dried (60 °C for 48 h) weekly, and at the end of the experiment, those samples were mixed to obtain a subsample which was used to determinate chemical composition [21–23]. Content of metabolizable energy (ME) was calculated with formula [24]. Thus, the chemical composition of diet was 93.9% dry matter (DM), 85.6% organic matter, 16.9% crude protein, 2.8 Mcal of ME/kg of DM, 37.2% neutral detergent fiber and 21.37% acid detergent fiber.

2.3. Feed intake, body weight and blood sampling

Daily amounts of feed offered and refused were weighed before the morning feeding to calculate dry matter intake (DMI). Also, BW was recorded as well as blood samples were taken individually at days 1 and 34 of experiment, just before the morning feeding. Blood samples were collected in 10 mL vacutainer tubes by jugular venepuncture, and then transported to the Animal Physiology Laboratory to be centrifuged at 3500 × g for 15 min at 10 °C. Serum was stored in 2-ml vials at −20 °C until its utilization to determine metabolite concentrations (glucose, cholesterol, triglycerides, urea and total protein) and thyroid hormones (triiodothyronine [T3] and thyroxine [T4]). Metabolite concentrations were analyzed with a blood auto-analyzer of liquid phase (Easy Vet, KONTROLab, Michoacán, Mexico), while thyroid hormone concentrations were measured with the ELISA technique using validated commercial kits (Monobind Inc., Lake Forest, CA, USA). The coefficients of variation between and within assay in the analyzed samples were 6.8 and 4.5%, respectively.

2.4. Evaluation of the reproductive tract and ovary

All ewe lambs were transported to the Meat Laboratory after