Animal Reproduction Science xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Effect of supplementing a diet with monensin sodium and *Saccharomyces Cerevisiae* on reproductive performance of Ghezel ewes

Leila Ahmadzadeh, Ali Hosseinkhani*, Hossein Daghighkia

Department of Animal Science, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

ARTICLE INFO

Keywords:
Blood variables
Fecundity rate
Feed additives
Reproductive hormones
Supplemental feed
Twining rate

ABSTRACT

Effect of supplementing a diet, in an attempt to enhance reproduction, with monensin sodium and *Saccharomyces cerevisiae* yeast on reproductive performance was investigated during the breeding season using 44 Ghezel ewes (body weight 56.97 ± 7.47 kg, age 2–5 years and body condition score (BCS) 2.5) which were allocated randomly in equal numbers to the four dietary treatments as follows: 1) Basal diet plus supplemental feed (450 g/ewe/d) plus monensin sodium (30 mg/ewe/d) (MS); 2) Basal diet plus supplemental feed (450 g/ewe/d) plus*Saccharomyces cerevisiae* yeast (4×10^9 CFU/ewe/d) (SC); 3) Basal diet plus supplemental feed (450 g/ewe/d) (FG); 4) Basal diet (only grazing on pasture, Control; G). Estrous synchronization of all ewes was done using controlled internal drug release (CIDR) and all ewes were mated with purebred Ghezel rams after CIDR removal. The results indicated that MS and SC treatments with 15 lambs had greater number of lambs than ewes of the other two treatment groups. Ewes in MS group with 50% twining rate had the greatest value followed by the FG, SC and G treatment groups ($P < 0.05$). The lambs from ewes in MS and SC groups were heavier in weight than those in FG and G treatments ($P < 0.01$). Blood sample analysis provided evidence that ewes in MS and SC groups had greater concentrations of 17β-estradiol (E2), progesterone (P4), blood urea nitrogen ($P < 0.05$), insulin, glucose, cholesterol and total protein ($P < 0.01$) than ewes of the other groups. These results indicated that using a diet for enhancing reproduction, including monensin sodium and *Saccharomyces cerevisiae* yeast in the breeding season could have beneficial effects on reproductive performance of Ghezel ewes.

1. Introduction

Ghezel sheep is one of the Iranian breeds, which is produced on the Sahand mountain rangelands. Lighvan cheese, which takes its name from a village, is produced by milk of this breed and is one of the most popular cheeses among Iranian people. Increasing demand for this cheese in Iran and low twining rate (about 11%, Sharifi et al., 2016) of this breed provides for the need for development of simple and applicable techniques for improvement of reproductive efficiency in this breed. Among the different methods, the “flushing” technique has been used widely to enhance the reproductive efficiency of sheep. This technique involves increasing the nutrient intake of ewes a few weeks before and after mating which can improve energy balance, body condition score, ovulation rate and thus lambing rate.

The use of feed additives containing live microorganisms or the metabolites, as well as antibiotics to manipulate rumen

* Corresponding author.
E-mail address: A.hosseinkhani@tabrizu.ac.ir (A. Hosseinkhani).

https://doi.org/10.1016/j.anireprosci.2017.11.013
Received 29 July 2017; Received in revised form 20 October 2017; Accepted 9 November 2017

fermentation and improve animal performance has increased during the last 2 decades (Sontakke, 2012). Supplementation of the "flushing" diet with some feed additives including probiotics (i.e., *Saccharomyces cerevisiae* yeast) and antibiotics (i.e., monensin sodium) can increase reproductive efficiency by directing rumen fermentation towards more propionate production and thereby more glucose and insulin or decreasing rumen degradability of protein. Information on how the incorporation of monensin and *Saccharomyces cerevisiae* and the interaction, affect reproductive performance in sheep is, however, limited. Additional investigation of feed additive roles in the Ghezel sheep feeding can increase the understanding of the physiological mechanisms that are affected that result in enhanced reproductive efficiency and fecundity rate. The purpose of the current study was to examine the effects of monensin sodium and *Saccharomyces cerevisiae* yeast on reproductive performance of Ghezel ewes.

2. Material and methods

2.1. Animals and experimental design

The present study was undertaken in the Agricultural Research Institute of University of Tabriz from August to February 2015. Iranian Ghezel ewes (n = 44; 56.97 ± 7.47 kg, 2–5 years old and BCS about 2.5) were allocated randomly to four equal groups (n = 11). All of the animals were grazing in the rangeland. Diets used in this experiment were isonitrogenous and isocaloric. Philips et al. (2002) recommended that "flushing" diets fed in attempts to enhance reproduction should provide 227–454 g of a grain supplement that contains at least 60% total digestible nutrients (TDN) and 10% crude protein. According to these recommendations, due to the poor vegetation of the rangeland where the ewes of this study grazed, the "flushing" diet was formulated so that it contained 80% TDN and 13% CP that were available for ewes at the rate of 450 g/ewe/day after ewes returned from pasture each evening of the study.

Designed treatments consisted of: 1) basal diet plus supplemental feed plus monensin sodium (30 mg/ewe/d) (MS); 2) basal diet plus supplemental feed plus *Saccharomyces cerevisiae* yeast *LEVUCELL SC20* (4×10^9 CFU/ewe/d) (SC); 3) basal diet plus supplemental feed (FG); 4) Basal diet (only grazing on pasture, Control; G). Diets of all animals were formulated according to National Research Council (1985) so that the diets of animals fed supplemental feed were according to "flushing" requirements and animals in the control group were only fed in amounts that ensured maintenance of their body condition. The three groups fed supplemental feed were housed in a separate barn and had access to the supplemental diet (barley grain, wheat bran, soybean meal, molasses, salt, vitamin and mineral supplement; 73%, 10.5%, 8%, 6%, 0.5%, 1%, 1% on kg DM basis, respectively). The method described by Thompson and Meyer (1994) was used for determination of ewes BCS. Sodium monensin and *Saccharomyces cerevisiae* yeast supplements were provided by BehroodAtrak Co. Iran and Lallemand Co., France, respectively. Monensin sodium contained in the supplement at 10% of the total amount of supplement as an active dose was applied at the rate of 30 mg/ewe/d according to the APVMA (Australian Pesticides and Veterinary Medicines Authority) recommendations. *Saccharomyces cerevisiae* yeast contained 0.4×10^9 CFU/g and 10 g yeast containing 4×10^9 CFU/ewe/d were added to the diet based on company recommendation. The *LEVUCELL SC20* consisted about 80% dry live cells and 20% dead yeast cells from the strain *CNCM I-1077 Saccharomyces cerevisiae* that did not remain viable after the drying process. All ewes received treatments for 5 weeks (2 weeks before and 3 weeks after mating). The stage of the estrous cycle of ewes was synchronized using a CIDR (EAZIBREED-NEWZELAND, each CIDR containing 0.3 g progesterone) that was in place for 14 days. At 48 h after CIDR removal and intramuscular injection of PMSG (400 IU/head), all ewes were mated with purebred rams with the same fertility and a ratio of one ram to two ewes.

2.2. Pasture sampling and chemical analysis

Pasture was located at Agricultural Research Institute of University of Tabriz and consisted of a mix of six predominant species including *Bromus Ramosus Huds*, *Cephalaria Microcephala Bioss*, *Festuca Glauca*, *Hieracium Piloselloides*, *Lolium Rigidum Gaulier*, *Vicia Villosa Roth*. Two paddocks were selected randomly and gridded into seven blocks for sampling. Before a new paddock was grazed, two transects and six plots were obtained by manual clipping at the soil level (3 cm). Samples of each plot were thoroughly mixed, dried and then sampled for further analysis. For evaluation of pasture content, dry samples were grounded through a 1-mm screen in a UDY cyclone-grinding mill (model 3010-030, UDY Corporation, Fort Collins, CO). Samples were analyzed for DM and CP content according to AOAC (2004). Digestible energy of pasture content was calculated by digestibility trials and metabolizable energy was estimated using *in vitro* gas production method (Fedorak and Hrudey, 1983). Pasture dry matter intake of animals was calculated by following equation (Molle et al., 2008): $y = 4.22\,x - 184.33$ where y = Dry Matter Intake (g DM/kg $BW^{0.75}$) and x = Dry Matter Digestibility (%) in which dry matter digestibility was calculated to be 56.82% using the acid insoluble ash method.

2.3. Laboratory analyses

Blood samples were collected from jugular vein puncture 4 h after feeding during four different stages: initiation of the experiment, 24 h before CIDR removal, 24 h after CIDR removal and 21 days after ram introduction. Sera were harvested by centrifugation for 12 min at 4000 rpm (Ependorf) and then each serum sample was divided into two 1.5 ml micro tubes and kept in −20 °C freezer until further measurements. Serum hormones were determined using an ELISA reader (STAT-FAX 3200, USA) set and micro plate enzyme-linked immunoabsorbent assay (ELISA) kits were used for determination of insulin (no. 2425-300 MonobindInc, USA), E2 (no. 4925-300A, MonobindInc, USA) and P4 (no. 4825-300 MonobindInc, USA). The intra-assay CV for the insulin, E2 and P4 were 8.54, 7.58 and 8.41% respectively. The inter-assay CV were 9.8, 9 and 12.1% for insulin, E2 and P4 respectively. Commercially