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Protein based flushing related blood urea nitrogen effects on ovarian response, embryo recovery and embryo quality in superovulated ewes

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ABSTRACT

The present study is the first report that evaluates effects of nutritional effects of flushing with differing diet crude protein ratios on blood urea nitrogen (BUN) levels, related some reproductive parameters and embryo quality in ewe. During mating season, before synchronization protocol ewes were fed on alfalfa hay and additive concentrate feeding as flushing. Intra vaginal FGA containing sponges applied for 12 days for the purpose of synchronization and pFSH was administered by 8 declining doses for the purpose of superovulation.

Uterus was flushed in the morning of the seventh day of mating and embryos were collected surgically. Collected embryos were qualified according to IETS criterion. There is no dependency found between BUN values measured at different days and at different diet crude protein concentrations. An increase in uterine pH levels due to increasing protein amounts was observed but this increase was not significant among groups. Ovarian function was evaluated by ovarian responses (CL + large follicle) showed difference between groups ($p < 0.05$) and the lowest protein intake group gave highest ovarian response. In addition, embryo recovery rates revealed difference between groups ($p < 0.05$) and it was observed that the lowest ovarian response group showed the highest rates of embryo recovery. It is concluded that, in some Anatolian native sheep breeds, the application of diet flushing with different crude protein concentrates influence ovarian responses and embryo recovery rates but has no effect on BUN levels; uterus physiology or embryonic quality.

1. Introduction

The present study aims to investigate the effects of nutritional protein flushing on BUN levels, some uterus physiological parameters, ovarian responses and embryonic quality in some native Anatolian ewe breeds during the breeding season. Nutrition affects all reproductive processes from gametogenesis to puberty and further in both female and male specimens [1]. A wide array of researches have been focused on revealing both environmental factors and mechanisms at play. While studies related to ewe are not sufficient, there are numerous studies conducted on dairy cows, focusing mostly on which relationship between diet protein levels and milk production; BUN levels and decreased fertility [2–7]. The general point of view attends the association between reproduction and nutrition from the frame of energy balance and research models were based on energy maintenance [1,8]. Also the majority of the studies [9,10] evaluating different protein concentrations indicate that increasing diet protein levels would result in decreased conception rates.

In natural conditions, survival of the species critically associated with seasonal feeding and reproduction. Along with the domestication, basically, reproduction capacity of animals always put forward with the aim to increase capacity. Thus new approaches to improve reproductive capacity have been the subject of new studies. Although it is mentioned especially with the ewe, flushing feeding before mating and seasonal reproduction is an important part of the reproductive management in seasonally reproducing animals. Researcher studied feeding flushing in ewe and cow mostly in terms of energy maintenance. They concluded in consensus that high and low energy diets are detrimental to oocyte and embryo development and related production processes [10–15]. But the effect and efficiency level of diet protein levels on reproductive physiology is remained elusive.

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Proteins and providing amino acids are vital for growth, metabolism, and reproduction. Ruminants have the ability to synthesize amino acids in rumen microflora and produce essential amino acids from non-protein nitrogen resources. Ruminants also have the ability to decrease protein loss by recycling urea [11]. But they also need structural proteins for urea cycle and microflora to work well.

The relative importance of flushing on reproductive efficiency illustrates the difficulty of determining protein and energy requirements for maximum reproductive performance. However, Torell DT et al. (1972) [9] concluded that notwithstanding protein and energy, on the basis of maximum live weight gain resulted in maximum reproductive performance. High urea in circulation and milk associated with excess protein intake and probably energy shortage [13]. On the other hand, increase in energy and protein in diet, raise nitrogen retention in ruminants and results in urea increase, which is one of the main actors of this research. Urea is a relatively small molecule (about 60 g/mole) that can freely move between cell membranes, therefore, circulation of urea between blood and other tissues is especially high [6]. Also urea is an important part of the protein metabolism and nitrogen cycle in ruminants. Again, it is clearly stated that increasing BUN levels are related to decreased fertility and embryonic loss [16]. Thus it can be said that direct effect of high protein intake on uterus may be detrimental for reproductive processes because of high urea. Toxic components of nitrogen metabolism, most particularly ammonium ions may affect sperm motion through oviduct; oocyte maturation; fertilization and early embryonic survival. It is reported that these effects may be modulated by uterine pH [10). By the way, McEvoy et al. [17] concluded that dietary changes which elevate ovulatory responses can be detrimental for embryo quality. In addition, it is accepted that high energy level effects oocyte and embryo quality may have negatively [18]. As a result, though there are many unclear definitions, it is clear that improving energy and protein intake by supplemental feeding is detrimental for oocyte and embryo. In comparison with more clear evidence of nutritional supplementation to enhance energy maintenance, protein based effect of reproductive efficiency is not clearly associated with BUN levels. Thus, the current study has two main objectives: 1) to test effects of different crude protein ratio supplemental feeding on reproductive efficiency directly and 2) to determine indirect effect of BUN on uterine physiology and reproductive efficiency.

2. Material and methods

The study was carried out during major mating season (late in summer and early in autumn) when most of the ewe manifest sexual activity in Turkey. The animals were maintained at the Animal Husbandry Unit of Selçuk University in Konya, Turkey which is located in Central Anatolia Region and at latitude 38° 1′ N, longitude 32° 30′ E and 1177 m of above sea level. Application procedure was sanctioned by Commission for Ethics on Animal Experiments of Selçuk University. Ewe breeds that are native to Anatolia (Daglıç, Herik, and Norduz) are kept under standard husbandry practices.

2.1. Experimental design

In the present study, 63 non-pregnant and cycling ewes of Anatolian strains aged 3–4 years and same body condition score (BCS:3) were used. Particularly the ewes that have undergone a successful pregnancy prior to the study were scanned for and selected. The ewes were randomly allocated in equal number ($n = 21$) to three different dietary groups. Also, the ewes were housed in slatted indoor housing and there was no direct and close male effect surrounding. The ewes were fed by dry alfalfa hay and sunflower meal before feeding flushing. With the onset of the study, in addition to ad libitum alfalfa hay, 700 g of concentrate mixture were provided per ewes per day as daily diet (nutritional composition of concentrate mixture shown in Table 1). Each group was allocated into one of the following diet groups depending concentrate mixture: (i) high protein diet (HP), (ii) medium protein (control) diet (MP) and (iii) low protein diet (LP). For optimal condition adequate open space was provided for all ewes. Feeding flushing procedure was maintained until embryo recovery.

2.2. Estrus synchronization and superovulation

The synchronization protocol was initiated 15 days after feeding flushing (see Table 2). To synchronize estrus, intra vaginal sponge containing 20 mg FGA were administered (Chronogest CR®, Intervet Productions SA., France) for 12 days [19,20]. Examination coupled with injection of a prostaglandin analog 0,392 mg (2 ml) tiaprost trometamol (Iliren®, Intervet) IM 72 h prior to sponge withdrawal. Superovulatory treatment was the same for all ewes and was initiated 4 days prior to mating. Each ewe was implemented with 200 mg pFSH (Folltropin-V®, Bioniche Animal Health, Canada) twice a day (morning and evening) in decreasing doses over a period of 4 days. The ewes were observed for estrous behavior by using a teaser rams for 30 min for every 6 h starting from 24 to 76 h after sponge withdrawal. Estrus positive ewes were filtered and immediately subjected to fertility-proven rams twice a day (morning and evening) for each. The vaginal mucosa of the ewe was controlled for ejaculation in order to assure that the mating is carried out. Ovarian responses and uterus physiology were evaluated on the 7th day of mating by laparotomy and the recovered cells were classified according to their quality.

2.3. Blood sampling and BUN analysis

Blood samples were collected respectively; at the beginning of feeding flushing procedure, at the beginning of synchronization; sponge withdrawal day; superovulation initiation day; on days of mating and surgical procedure day. In order to overcome stress on ewe, food and fresh water provisions were halted until the end of applications. Blood samples were collected from jugular vein of ewes into 10 ml plain vacuum tubes (Vacutainer®, Becton Dickinson, England). All samples were labeled and centrifuged at 3000 × g for 5 min after an hour following sampling. Extracted serum was deposited into 1.5 ml of Eppendorf tubes, labeled and stored at −20 °C until the analysis of urea nitrogen. Serum urea nitrogen concentration was measured using BT-3000 plus

Table 1
Nutritional compositions of feed.

Test	Group 1	Group 2	Group 3
Dry matter	%88,4	%88,6	%89,3
Ash	%7,4	%7,3	%7,1
Crude protein	**%12,17**	**%15,02**	**%17,92**
Acid detergent fiber (of dry matter)	%8,3	%11,6	%13,27
Neutral detergent fiber (of dry matter)	%19,52	%25,03	%26,8
[a]TDN (of dry matter)	%67,90	%65,07	%67,04
Fat (ether extract, of dry matter)	%2,46	%2,6	%3,98
Metabolic energy (Mcal/kg)	%2,47	%2,44	%2,49
Calcium	%1,44	%1,33	%1,15
Chloride	%0,3	%0,3	%0,3
Magnesium	%0,3	%0,4	%0,4
Phosphor	%0,2	%0,2	%0,3
Potassium	%0,7	%0,9	%0,8
Sodium	%0,2	%0,2	%0,2
Sulphur	%0,1	%0,2	%0,2

Bold indicates especially significant for the purpose of research's main topic.
[a] TDN: Total Digestible.