Arabi lamb's rumen morphological changes in response to different level of treated straw with urea supplement

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Abstract

The goal of this study was to use histomorphometry evaluation in combination with treated straw with urea in addition to mixed rations to show the ontogenesis of the rumen in Arabi lambs. This study was conducted in the animal field/ College of Agriculture/ University of Basra to show the effect of using straw treated with urea in different proportions on some anatomical characteristics of rumen of Arabi male lambs. In this study, (16) Arabi male lambs with ages ranging between (3-4 months) and average weight of (23.5 kg) were used. Lambs were randomly distributed into four treatments, with four animals for each treatment. The concentrate feed was mainly barley (55%), wheat bran (43%), vitamins and minerals (1%) and salt (1%). Lambs were fed 3% of their live body weight. The first group was fed 100% concentrate feed, the second group 90% concentrated feed + 10% straw treated by urea, the third group 80% concentrated feed + 20% straw treated by urea, and the fourth group: 70% concentrated feed + 30% straw treated by urea. Significantly higher ($P < 0.05$) pH was recorded in all the treated groups after 8 h of feeding. Similarly, the brightness (L*), redness (a*) and yellowness ((b*) of rumen was significantly higher ($P < 0.05$) in treated groups. The papillary length, width and surface area of rumen were significantly ($P < 0.05$) high in treated straw supplemented groups. The structural properties of the rumen were enhanced by a diet plan mainly concentrate with the inclusion of treated straw with urea at the level of the indicated procedure.

Keywords: Arabi lamb, rumen, urea supplement

Introduction

Animal welfare, as well as animal production, is influenced by feeding practices (Di Giancamillo *et al*., 2003). To meet the growing needs for lean meat in the current days, sheep and goats are fed a diet that may endanger their wellbeing. In many countries, the production of mutton has also transitioned from a conventional approached to a new intense rearing system, in which animals are fed a highly concentrated diet to assure rapid growth. The transition from suckling to weaning is a sensitive time in which young lambs are subjected to a high-energy diet, which can lead to severe complications such as rumen acidosis and abnormal structural and functional development of the rumen wall in lambs (Cavini et al., 2015), putting animal welfare at risk. The development of rumen papillae is substantially dependent on the synthesis of volatile fatty acids (VFAs) in ruminant, particularly butyrate and propionate, which regulate the rumen mucosa in sheep (Mach et al., 2006; Alvarez-Rodrguez et al., 2010, Franco et al., 1992). Small ruminants are classified as transitional consumers, lying in between pasture browsers and concentration feeders (Garcia et al., 2012). As a result, a formalized ration is required to allow growing lambs to adjust to the new diet program in order to assure rapid growth without the risk of post-feeding issues.

In different regions of the world, various fattening strategies are popular, based on farm infrastructure, consumer preferences, and economic variables. Pasture and silage, or concentrate feed and forage, are the most frequent fattening systems (Bodas et al., 2014). The concept of total mixed rations (TMR) has been effectively used to boost farm animal weight gain and milk output (Amaral et al., 2005; Nissanka et al., 2010; Shekarchian,2012). Breeders are more willing to reduce the concentrate feed and switching to a roughage diet as a result of the dramatic price rise of feedstuffs and the resulting ruminal difficulties (Bodas et al., 2014). Although it is widely proven that a concentrate-based diet promotes higher growth (Casasus et al., 2012), it has been challenged for fattening lambs and calves by causing rumen acidosis, laminitis, and parakeratosis (Bodas et al., 2014; Blanco et al., 2015). Although a forage-based diet can achieve similar effects, it is related to improved feed consumption and a lower feed utilization (Casasus et al., 2012). Cooke et al., (2004); Mach et al., (2006); Tufarelli et al., (2011and 2012); Alvarez-Rodrguez et al., (2012); Bodas et al., (2014) found improved rumen condition with appropriate pH and papillary development in ruminants when using concentrate-based TMR with a particular degree of forages. Roughage consumption enhances rumen motility, stimulates rumination, and promotes chewing and salivation in calves (Van Ackeren et al., 2009), probably because to the availability of functionally useful fiber (Beauchemin and Buchanan-Smith, 1989; Allen, 1997a, b). Furthermore, in fattening animals fed a 100% TMR-based diet, discoloration of the rumen has already been reported in lambs (Blanco et al., 2015), which might also lead to negative lamb welfare.

The ability of ruminants to transform coarse fiber into extremely nutritious feed has sparked interest in the anatomical and functional studies of their stomachs (Franco et al., 2012). Ruminants' stomachs are divided into four champers, each of which serves a particular purpose and has its own anatomical traits. The rumen is the most significant compartment physiologically because it aids in the breakdown of cellulose-based diet and creates a favorable environment for microbial digestion (Garcia et al., 2012). Numerous morphological studies have been conducted on the rumen of sheep, with the majority of the studies concentrating on processes associated with the progressive stage (Franco et al., 1992, 2012; Garcia et al., 2012). However, data on the impact of feeding system on the integrity of the rumen in sheep is limited.

When 50 percent of the lamb's estimated net energy needed was supplied in the form of short-chain fatty acids at physiological concentrations, papillae length rose (Lane and Jesse, 1997). The amount of VFA produced surpasses the ability of the ruminal papillae to absorb it, it builds up in the rumen, lowering the ruminal pH and causing the rumen epithelium to atrophy (Wang et al., 2009).

We hypothesized in this study that treated wheat straw with urea given in diverse patterns will impact rumen properties in Arabi lambs in distinct ways.

Materials and Methods

This study was conducted in the animal field of the College of Agriculture/University of Basra. The study included a growth experiment and a digestion experiment. The study included 12 Arabi lambs after weaning at the age of 3-4 months, their weights mean 22 kg. Lambs were divided into three treatments randomly after numbering with plastic numbers. Four animals for each treatment were placed in a half-shaded barn. Water was given freely for the duration of the study. The feed was provided on the basis of 3% of the live body weight, and the quantities provided were adjusted based on the new weight for each treatment every two weeks. The feed was provided on two meals at eight o'clock in the morning and at four o'clock in the afternoon, and the amount of feed provided and remaining daily was calculated for the purpose of calculating the quantity of feed intake. Green fodder provided as freely available.

Table (1). Feed composition and chemical analysis of control group

Nutrient	Percent
Barley	55.00
Wheat bran	43.00
Salt	1.00
Vitamin and minerals	1.00
Dry matter	89.60
Crude Protein	13.00
Crude Fiber	7.96
Ash	3.51
Nitrogen free extract	50.82

At the beginning of the experiment, the lambs were fed an introductory period for ten days and then weighed to record the initial weight. The experimental treatments were the control group without any addition (100% concentrate). The second treatment, 90% of control diet plus 10% treated wheat straw with urea. The third treatment, 80% of control diet plus 20% of treated straw. The fourth treatment, 70% of control diet plus 30% treated straw. Table (1) showed the composition of concentrated feed with chemical component.

Straw treatment

The straw was purchased from the local markets in Basra Governorate. A total of 100 kg of straw was spread on a large piece of nylon, about 10 cm thick. 40 liters of fresh water and 4 kg of urea were dissolved in it and stirred well until it melted completely. The solution was sprayed directly on the straw by a sprinkler, and the straw was constantly stirred during the spraying so that the solution would reach completely all parts of the straw in order to ensure the homogeneity of the treatment. The treated straw placed in a 10 kg nylon bags and tightly controlled to prevent the leakage of ammonia resulting from the decomposition of urea. The treated straw was left for 21 days at room temperature, then the bags were opened and the straw spread on the nylon in the air with stirring for the purpose of not leaving any undesirable smell, and then it was mixed with the concentrate to feed the lambs in the proportions mentioned in the study.

At the age of six months, twelve male lambs were slaughtered after 16 h fasting. At around 5 cm from the longitudinal pillar of each lamb, small pieces (5x5 cm) were taken from the dorsal sacs of the rumen. Specimens were fixed in 10% phosphate-buffered formalin for 24 hours, then routinely processed, embedded in paraffin, sectioned at a 4 m thickness, stained with Hematoxylin Eosin (H&E), and viewed with a microscope attached to the camera (Olympus DP72 Microscope Digital Camera; Olympus NV, Aartselaar, Belgium). Rumen tissue samples were collected for analysis of papillae length (PL), papillae width (PW), and rumen wall thickness (RWT) according to Lesmeister *et al* (2004). The length of papillae was defined as the distance from the tip to the base of the papillae while papillary width was defined as the average width of the base, middle and tip of papillae. The line representing the length started at the base of the lamina propria and extended to the tips of the papillae. For papillae width, the lines were drawn on the widest part at right angle to the lines representing length. Papilla height and width data were used to calculate papilla surface area [$2\pi x(W/2) \times L$], where W = papilla width and L = papilla length. Surface area was estimated considering them cylindrical in shape with one closed end. In addition, measured width was assumed equal to the diameter of papillae (Alvarez-Rodrigues et al., 2012).

Digestive tracts were harvested, emptied, and rinsed with cold water, and rumen tissue samples were collected for analysis of papillae length (PL), papillae width (PW), and rumen wall thickness (RWT).

Results and Discussion

The present study provides an overview of macroscopic and microscopic morphological changes of the rumen papillae in weaning lambs at 90 days of age to the age of 180 days and presents the first data showing the effect of postweaning concentrate build-up and the addition of on rumen morphology. The addition of treated straw with urea significantly(P<0.05) altered all color measurements (Table, 2). In compared to the control group, all treatments with urea showed significantly (P< 0.05) increased brightness values, with T3 recording the greatest value (43.90) and control recording the lowest (38.38). T1 had the greatest redness value (5.03), whereas control and T3 had the lowest and most similar values (3.73 and 3.84 respectively). In terms of yellowness, all treated groups varied substantially (P<0.05) from the control group. While the distinctions between them are minor.

The dark brown color of lambs fed treated straw with urea has vanished in the current investigation. Animals fed a concentrated diet had a deeper rumen color, which is linked to rumen mucosa stiffening caused by low pH. (Blanco et al., 2015). Alfalfa, on the other hand, produced a light brown tint. Blanco et al. (2015) and Alvarez-Rodrguez et al. (2012) obtained similar observations.

Table (2). Effect of different level of concentrates: treated straw on the color of rumen tissue of weaned Arabi lambs

Treatments	Color components		
	Brightness (L)	Redness (a)	Yellowness (b)
Control	38.38d	3.73c	9.13b
T_1	41.87c	5.03a	9.94a
T_2	42.79b	4.09b	10.04a
T_3	43.90a	3.84c	9.83a
SEM	0.27	0.57	0.49

- **Means with different superscript in each column differ significantly at P<0.05**
- **Control=100% concentrate, T1=90% control+10% treated straw, T2=80% control+20% treated straw, and T3=70% control+ 30% treated straw**

The histological changes in the control and treatment groups differed significantly (P<0.05), as seen in table 3. Length of papillae associated significantly (P<0.05) with different treatments. The papillae of all treatment groups were longer, higher width and bigger surface area than those of the control group. The T2, T_2 and T3 group had a larger surface area (40.73, 42.19 and 42.81 mm^2 respectively) than the control group (33.38 mm^2). Various dietary groups displayed similar papillae widths (1.57, 1.61 and 1.63 mm for T1, T2 and T3 respectively).

Table (3). Effect of different concentrate: treated straw on the dimension of papillae of weaned Arabi lambs

Treatments	Papillae dimension		
	Length (mm)	Width (mm)	Surface area (mm^2)
Control	7.86b	1.35b	33.38b
T_1	8.27a	1.57a	40.73a
T_2	8.36a	1.61a	42.19a
T_3	8.37a	1.63a	42.81a
SEM	0.26	0.13	4.21

- **Means with different superscript in each column differ significantly at P<0.05**
- **Control=100% concentrate, T1=90% control+10% treated straw, T2=80% control+20% treated straw, and T3=70% control+ 30% treated straw**

In the control and T1 groups (table, 4), the stratum corneum was connected with a thick layer of epithelium (0.16 and 0.17 mm, respectively). In lambs given both treated straw and concentrate, the thickness of the stratum corneum was significantly ($P<0.05$) reduced. T2 and T3 recorded the lowest values (0.12 mm for both treatments). Values of Lamina propria of T3 was the lowest (0.91 mm), whereas, control, T1 and T2 revealed nearly similar values. T2 showed the highest ($P<0.05$) value of submucosa (2.04 mm), the other groups showed similar values but less ($P<0.05$) than that of T2.

Table (4). Effect of different concentrate: treated straw on the dimension of dimension of stratum corneum, lamina propria and submucosa of papillae of weaned Arabi lambs

Treatments	Stratum corneum (mm)	Lamina propria (mm)	Submucosa (mm)
Control	0.16b	1.09a	1.60b
T_1	0.17a	1.17a	1.67b
T_2	0.12c	1.07a	2.04a
T_3	0.12c	0.91b	1.72b
SEM	0.004	0.04	0.071

- **Means with different superscript in each column differ significantly at P<0.05**
- **Control=100% concentrate, T1=90% control+10% treated straw, T2=80% control+20% treated straw, and T3=70% control+ 30% treated straw**

Microbial fermentation products (mostly butyrate and propionate) and physical stimulation stimulate the growth of rumen papillae (Flatt et al., 1958; Rickard and Ternouth, 1965), whereas physical properties of feed bulk control the size and musculature of the rumen (Harrison et al., 1960; Rickard and Ternouth, 1965).

Alvarez-Rodríguez et al., (2012) demonstrated that forage availability had no influence on papillae height or width, but it did have an effect on papillae surface area, which was lower in alfalfa-fed lambs than in concentrate-fed lambs.

Ad libitum grazing lambs with creep feeding did not result in increased rumen epithelial development when compared to high-concentrate feeding. However, rumen papillae formation was enhanced in calves given more concentrate as well as in grain-challenged wither lambs given a subacute ruminal acidosis (Zitnan et al., 2005; Mach et al., 2006 and Odongo et al., 2006).

Butyrate and propionate control of IGF-I synthesis, which increases papillary size and density, could be one of the underlying mechanisms for this reaction (Shen et al., 2004).

The keratinization of the papillae, which was also observed in this study, leads to decreased VFA absorption, which is necessary for the rumen's muscular growth and development (Van

Ackeren et al., 2009). From the findings of this study, it can be concluded that allowing lambs full access to a specific amount of treated straw may have a positive influence on feed intake when compared to the control group, resulting in enhanced dry matter intake and weight gain in the respective groups.

When dietary starch was set at 46 percent of dry matter intake for 35 days, rumen papillae growth and wall thickness were affected in young goats (Wang et al., 2009). The starch level of the concentrate employed in this study (42.5%), on the other hand, fostered a mix of rumen fermentation end-products that favor papillae formation at the expense of rumen muscle layer thickness. When the slaughter body weight and average daily gains during the raising period were taken into account (Alvarez-Rodrguez et al., 2010), the dry matter intake calculated by Cannas et al. (2004) for the current growing lambs reached a maximum value of 0.9–1.0 kg/day. This would indicate that during the rearing phase, the intake of easily fermentable carbohydrates did not surpass the aforementioned level.

Concentrate-fed lambs, on the other hand, acquired greater rumen parakeratosis than alfalfa grazing lambs, as demonstrated by the color of the ruminal epithelium and observations. This situation is characterized by an increased number of cell layers in the stratum corneum, comprised of cornified keratinocytes near to the lumen that operate as an additional protective barrier (Hinders and Owen, 1965). (Baldwin, 1998). Parakeratosis has recently been linked to the loss of adherent microbial colonization of the ruminal epithelium (Steele et al., 2012).

The rumen's mucosal lining is made up of various papillae, which are the primary absorbents of nutrients and water required for proper growth and development (Graham and Simmons, 2005), leading to the conclusion that the longer and wider the papillae, the larger the surface area for absorption and the more effectively nutrients can be uptake. The major sign that can serve as the standard diagnostic way to measure the influence of nutrient addition to fodder on rumen growth is the thickness of the papillae (Lesmeister et al., 2004). The rumen epithelia are recognized to be important for short-chain fatty acid ingestion (Kramer et al., 1996; Penner et al., 2009).

Animals fed roughage in addition to hay have a faster rumen development, which helps a process that affects the rumen's mass, volume, and muscle layer thickness compared to those fed only hay (Baldwin et al., 2004; Khan et al., 2011). On the other hand, Costa et al. (2019) found that roughage or not has no effect on the length of the papillae. However, roughage promotes and develops the rumen muscle, although the thickness of its muscle layer is not affected by the growth of the rumen epithelia (Arne and Ilgaza, 2021).

The sudden increase in concentrate allowance caused a transitory discrepancy in fermented organic matter intake, influencing the development of the rumen papilla. With the rapid rate of rise in concentrate allowance, the group's rumen papilla surface area and width increased at a faster pace. The concentration treatment had no effect on the thickness of the papillae or the thickness of the epithelium, contrary to our expectations (Dieho et al., 2016).

In conclusion, current research examines the morphometric changes of the rumen papillae in lambs over the growth phase, as well as the impact of roughage level on rumen anatomy. Roughage level in the feed of Arabi lambs significantly affected papillae height, width and surface area. The level of 20% or more decreased Stratum corneum. However, increased submucosa significantly vs 0 or 10% and 30% of roughage in the diet.

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