Determinating the relationship between starch level and acidosis in high starch containing diets in lambs

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SUMMARY

The aim of this study was to determine the effects of high starch levels in the diet on the formation of subacute acidosis in lambs and to determine the ideal starch level in lamb fattening diet. Three different diets with starch content of 25% (1st Ration-R1), 30% (2nd Ration-R2) and 32% (3rd Ration-R3) were prepared to induce subacute rumen acidosis to achieve this purpose. These diets were fed to 6 Akkaraman lambs, aged 11-12 months, with a body weight of 30-35 kg, in a replicated 3x3 Latin Square design to determine daily nutrient consumptions and digestibility of nutrients. Rumen fermentation parameters (pH, organic acid and ammonia nitrogen) were determined in the rumen fluid sampled at 0, 2, 4, 6, 8 hours post-feeding in each period. Blood samples were also taken at 0- and 6-hours post-feeding in order to determine blood gases and blood biochemistry parameters. Blood glucose, total protein, triglyceride, urea, creatinine and albumin, pH, pCO_2, HCO_3 and base clearance were measured in these blood samples. It was observed that the daily nutrient consumptions of animals decreased in parallel with the increases in starch in the diets ($P<0.05$). Similarly, in parallel with the increase in the starch level of the diet, there was a general tendency to decrease in digestion of nutrients other than starch ($P=0.08$). There was no statistical difference in general between rumen pH, total organic acid, organic acid ratios, only rumen ammonia levels were found to differ between the groups before and immediately after feeding (2nd hour) ($P<0.05$). While blood pH values and HCO_3 values decreased after feeding, no significant change was observed in other parameters. Among the blood biochemistry parameters, only creatinine decreased statistically ($P<0.01$) in the R1 group after feeding, and no difference was observed between other parameters. There was no significant difference among the groups in any of the blood parameters. It can be concluded that increasing the starch level above 25% in the diets of lambs adversely affects the nutrient consumption and nutrient digestion of the animals in general, and also reduces the blood pH and HCO_3 levels, thus creating a risk of subacute acidosis.

KEY WORDS

Lambs; starch; digestibility; rumen fluid; acidosis.

INTRODUCTION

It has been stated that there has been an increase of nearly 85% in the number of sheep and goats in Türkiye between 2010 and 2020 (1). According to TUIK data, 1 million 785 thousand 952 tons of red meat was produced in Türkiye in 2020, and this figure is estimated to be 1 million 952 thousand 38 tons in 2021 with an increase of 9.3%. In this context, beef production increased by 8.9% compared to the previous year and reached 1 million 460 thousand 719 tons, and sheep meat production increased by 11.7% to 385 thousand 933 tons (2). It has been reported that in order to reduce the red meat deficit in Türkiye, it is necessary to produce high quality lamb carcasses at low cost and to offer them for public consumption (1).
Considering that the feed cost can reach up to 70% in a livestock enterprise, it is essential to keep the diet costs as low as possible for a sustainable livestock. In order to reduce feed costs, cheaply produced cereal grains such as barley and wheat, are used in the diets. However, considering the rapid fermentation of the starch they contain, the rate of inclusion in the diet should be considered. Otherwise, it may cause acidosis, which is an important metabolic disease for animal. This leads to damage to the rumen, resulting in production and financial losses. Sugar and starch, which are non-structural carbohydrates in the diet, can be fermented to volatile fatty acids as well as to lactic acid (3, 4). Since sugars in the form of disaccharides are also converted to glycogen, they form less lactic acid than starch, and the risk of acidosis is relatively low compared to starch. Pectic substances contribute little to the formation of acidosis due to their weak fermentation properties at low pH. Therefore, it would be a correct method to prepare a diet by looking at the fermentation characteristics rather than the amount of these carbohydrate types, which have different effects on the fermentation characteristics and rumen pH (5, 6).
As long as starch-rich feeds are given to ruminant animals by getting used to it, it does not cause a problem up to a certain level. However, if the starch level in the diet rises above a certain level, the formation of acidosis is inevitable. Generally, the optimum starch level for dairy cow diets is recommended as 23-30% in DM, depending on the roughage level of diets (7). However, there are not many studies on optimum starch levels

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for fattening lamb diet in the literature. It is well-known that it is very important to know the required maximum starch level in order to prevent sub-acute acidosis cases in lambs, which are thought to be very important in fattening lamb diet.
The aim of this study was to determine the effects of high starch levels in the diet on the formation of subacute acidosis in lamb and to determine the ideal starch level in lamb fattening diet.

MATERIAL AND METHOD

Six Akkaraman lambs, aged 11-12 months, with a live weight of 30-35 kg, were used in the study. Lambs were canulated approximately 1 week after lambs were brought to the study area. The trial started approximately 1 month after the cannulas were inserted into the rumen o lambs.
During the trial period (15 days), the animals were individually housed in metabolism cages. Animals had free access to feed, vitamin-mineral blocks (500,000 IU Vitamin A, 100,000 IU Vitamin D3 per 1 kg, Vitamin D3, 150 mg Vitamin E, 3,000 mg Niacin, 4,000 mg Phosphorus, 6,250 mg Calcium, 5,000 mg of Iron, 2,000 mg of Zinc, 500 mg of Manganese, 500 mg of Copper, 12,000 mg of Magnesium, 100 mg of Iodine, 100 mg of Cobalt, 1,500 mg of Aroma) and fresh water. At the same time, liquid-permeable feces collection bags were fitted to cover the rump, tail, anal region and part of the abdomen of each lamb in order to collect the feces of the animals during the experiment.
Diets containing 25% (R1), 30% (R2) and 32% (R3) starch were prepared to induce rumen acidosis and fed to animals throughout the experiment. The nutrient contents and botanical compositions of the diets are given in Table 1. Based on chemical analysis, starch levels of R1, R2 and R3 were 25.58%, 30.55% and 32.96%, respectively.
Each of the prepared diets was fed to lambs in 3 periods of 15 days, with 10 days of adaptation to diets and 5 days of sampling. In each period, a diet was given to 2 lambs and fed in a replicated 3x3 Latin Square design. Feed was offered ad libitum at 08.30 in the morning and 20.30 in the evening. In order to determine their nutrient consumption, the amount of feed to be given to the animals in the last 5 days of each period was determined, at the end of 5 days, the remaining feed from the previous ration was weighed and a sample was taken for nutrient analysis.
Feces accumulated in the fecal collection bags were taken from the bags daily from 10 to 15.th days of each period (for 5 days), weighed and stored in cold chain rules. At the end of each period, after the total feces (feces collected for 5 days) samples of each animal were mixed homogeneously, an average of 100 g in samples were taken for analysis. All fecal samples were ground after drying in an oven at 65°C for 24 hours before chemical analysis.
Rumen fluid was taken from the cannulas of the animals in each period with the help of a manual pump and probe at 0, 2-, 4-, 6- and 8-hours post-feeding on the last day of each sampling period. Twelve ml of rumen fluid was immediately taken into test tubes with 2 ml of hydrochloric acid (HCl) solution diluted with 1/1 distilled water and frozen until analysis of organic acids. Ammonia nitrogen (NH_3-N) analysis of rumen fluids was performed on the same day. The pH of the rumen fluids was immediately measured with a digital pH meter (LaMotte DHA3000, USA) at sampling site.
Blood samples were taken from the animals for blood gas and biochemistry analyzes on the 15th day of each period, just before feeding (0. hour) and at 6-hour post-feeding.
Ammonia nitrogen was determined by the Kjeldahl method in the supernatants obtained by thawing and centrifuging the rumen fluids frozen during the experiment (8). Organic acid analyzes of rumen fluid in these supernatants were also carried out in the gas chromatography (Shimadzu GC-2010,

Table 1 - Botanical and nutrient content of the diets used in the study, %DM. (Mean ±SD).

Feedstuffs	R1, %DM	R2, % DM	R3, % DM
		Botanical Composition	
Ground Alfalafa hay	30.48	20.09	6.04
Wheat straw	-	5.85	13.76
Barley	18.44	22.42	24.67
Corn	20.35	24.16	26.14
Sunflower meal	26.55	26.25	28.02
Lime stone	1.08	1.23	1.37
Bypass fat	3.1	-	-
		Nutrient content	
DM	93.14	93.52	92.82
OM	94.88	94.36	95.60
CP	16.21	16.50	16.81
NDF	37.50	36.65	37.91
ADF	20.46	20.75	20.81
C	15.60	15.33	15.19
EE	2.27	2.45	2.49
Starch	25.58	30.55	32.96

Japan) in the laboratories of Kırıkkale University Scientific and Technological Research Application and Research Center (KÜBTUAM). Lactic acid analyzes in rumen fluids were carried out in Kırıkkale University Scientific and Technological Research Application and Research Center Directorate (KÜBTUAM) laboratories.

For pH measurement in feces, the feces collected daily throughout the study were mixed homogeneously and 3 replications from each animal feces, as stated by Verlinden et al. (9), after diluting 1 unit of feces with 9 units of distilled water, the feces were mixed. Their pH was measured with a digital pH meter (LaMotte DHA3000, USA).

The nutrient contents of the feedstuffs and orts samples were analyzed for ash, organic matter (OM), ether extract (EE), crude protein (CP), acid insoluble nitrogen (ADIN-N) according to AOAC (10), crude fiber (CF), (11), neutral detergent fiber (NDF; Van Soest and Robertson (12)) and acid detergent fiber (ADF; Goering and Van Soest (13)). Determination of starch in feeds and feces was carried out polarimetrically as specified in TS ISO 6493 (14).

Blood samples taken from animals were centrifuged at 3000 rpm for 10 minutes and serum was obtained. Glucose (GLU), total protein (TP), triglyceride (TG), urea, creatinine (CREA) and albumin (ALB) values were measured in this serum with an autoanalyzer (Mindray BS-2000M, China). pH, pCO2, HCO3, BE (Base excess), hematocrit, sodium (Na), potassium (K), calcium (Ca), chlorine (Cl), glucose (GLU) and lactate analyzes were performed in heparinized blood samples using blood gas device (Siemens Rapidlab 1265, Germany).

Statistical Analysis

The data were analyzed as replicated using 3x3 Latin Square trial design using the GLM procedures of the SAS statistical program (15). Tukey t-test was applied to determine the difference between the groups. Independent t-test was used for hour-to-hour comparisons of blood gas and biochemistry data.

RESULTS

In the study, the amounts of daily nutrient intakes and digestion rates of the diets fed to lambs are given in Table 2. The DMIs and DM digestion rates of sheep consuming R1, R2 and R3 diets were 2421.58gr (89.38%); 2086.90gr (86.58%) and 1627.44gr (85.74%), respectively. DMI was highest in lambs fed R1 and lowest in R3 diets among diets. Similar to dry matter intake, intake of all other nutrients in lambs was statistically different (p 0.05).

However, although DM digestion rates (89.38%, 86.58% and 85.74%, respectively) (p= 0.08) and OM digestion rates (89.30%, 86.45% and 85.65%, respectively) were not statistically significant, there was a trend to be different (p= 0.08). Similarly, a trend has been detected in digestion of other nutrients other than starch digestion (p= 0.08). The feces pH of animals consuming R1, R2 and R3 diets were 6.76, 6.71 and 6.60, respectively. The pHs among the diets were not statistically significant (p=0.12), but the pHs decreased proportionally with the increasing grain and starch levels in the diets.

R1: 25% starch diet, R2: 30% starch diet, R3: 32% starch diet, DMI: Dry Matter Intake, OMI: Organic Matter Intake, CPI: Crude Protein Intake, NDFI: Neutral Detergent Fiber Intake, ADFI: Acid Detergent Fiber Intake, STI: Starch Intake, DMD: Dry Matter Digestion, OMD: Organic Matter Digestion, CPD: Crude Protein Digestion, NDFD: Neutral Detergent Fiber Digestion, ADFD: Acid Detergent Fiber Digestion, STD: Starch Digestion, a, b, c, d: Different letters on the same line show statistical difference (p 0.05).

Rumen fluid parameters are given in Table 3 according to diet and hours.

In the measurements made every 2 hours post-feeding, rumen pH values were similar between the groups (P>0.05). Among the fermentation parameters, there was a difference between the groups in the rumen ammonia-N level at 0 and 2 hours, lactic acid at the 8th hour, and the ratio of acetic acid to total VFA only at the 6th hour post-feeding (P<0.05), while no difference was observed in other fermentation parameters.

The blood gas values of the blood samples taken from the animals in the trial are given in Table 4 according to the hours and diets. Among the results of blood gas values between diets, only the 6th hour post-feeding hematocrit values were statistically significant (p 0.05). Significant differences were observed in the blood pH of the animals in R2 and R3, in pCO2

Table 2 - Amount of nutrients consumed, g/day and digestion rates of nutrients, %. (Mean ±SD).

Intake, %DM	R1	R2	R3	*p*
DMI, g	2421.58^{a}±219.23	2086.90^{ab}±220.35	1627.44^{b}±79.48	*0.03*
OMI, g	2297.59^{a}±208.07	1969.20^{ab}±207.92	1555.84^{b}±75.98	*0.03*
CPI, g	392.54^{a}±35.55	344.34^{ab}±36.36	273.57^{b}±13.36	*0.04*
NDFI, g	908.09^{a}±82.24	764.85^{ab}±80.76	616.96^{b}±30.13	*0.03*
ADFI, g	495.45^{a}±44.87	433.03^{ab}±45.72	338.67^{b}±16.54	*0.03*
STI, g	637.55^{a}±67.32	619.44^{ab}±56.10	536.41^{b}±26.20	*0.03*
DMD, %	89.38±0.48	86.58±1.07	85.74±1.47	*0.08*
OMD, %	89.30±0.47	86.45±1.14	85.65±1.46	*0.08*
CPD, %	86.37±0.59	86.42±0.97	83.53±1.29	*0.09*
NDFD, %	80.10±0.85	75.07±1.68	77.08±1.19	*0.08*
ADFD, %	82.07±0.89	76.40±1.95	77.01±2.25	*0.08*
STD, %	92.61±0.65	92.33±0.52	92.16±0.30	*0.82*
Feces pH	6.76±0.05	6.71±0.04	6.60±0.06	*0.12*

Table 3 - The pH, NH_3 and VFA values of rumen fluids taken from animals. (Mean ±SD).

Hours	Diet	pH	NH_3 mg/dl	AA, mmol/L	PA, mmol/L	BA, mmol/L	LA, mmol/L	Total UYA, mmol/L	AA, % Total UYA	PA, % Total UYA	BA, % Total UYA	LA, % Total UYA
0	R1	5.78±0.07	8.49^{b}±0.33	58.53±3.31	18.77±1.97	14.24±1.18	2.90±0.44	94.45±3.96	61.97±0.89	19.87±1.45	15.08±0.53	3.07±0.31
	R2	6.00±0.12	9.56^{a}±0.29	57.32±3.78	18.85±2.07	14.73±1.87	2.82±0.44	93.72±5.64	61.16±1.08	20.11±0.98	15.72±0.95	3.01±0.26
	R3	5.94±0.11	9.70^{a}±0.26	58.57±2.16	19.02±1.07	15.44±0.25	2.85±0.22	95.88±1.82	61.09±0.76	19.84±0.70	16.10±0.22	2.97±0.15
	p	*0.3*	*0.001*	*0.07*	*0.51*	*0.81*	*0.99*	*0.1*	*0.26*	*0.51*	*0.58*	*0.76*
2	R1	5.51±0.06	11.39^{a}±0.22	58.90±3.83	21.99±1.19	15.41±1.25	3.01±0.21	98.31±5.26	59.91±0.47	22.37±0.73	15.67±0.47	3.06±0.15
	R2	5.60±0.13	10.16^{b}±0.34	59.69±2.66	20.14±2.97	16.52±1.47	3.32±0.29	99.68±5.22	56.88±1.41	20.20±1.04	16.57±0.71	3.33±0.13
	R3	5.60±0.07	10.65^{ab}±0.17	58.46±6.12	19.03±1.86	14.35±0.91	3.16±0.41	95.99±8.21	60.90±1.14	19.82±0.58	14.95±0.38	3.29±0.35
	p	*0.73*	*0.01*	*0.57*	*0.09*	*0.21*	*0.79*	*0.33*	*0.14*	*0.14*	*0.53*	*0.63*
4	R1	5.47±0.04	11.32±0.55	56.36±4.54	17.49±1.89	15.99±1.58	2.24±0.27	92.07±7.53	61.21±0.73	19.00±1.10	17.37±0.41	2.43±0.13
	R2	5.58±0.10	10.53±0.31	57.06±6.03	17.56±2.70	15.93±1.15	2.70±0.34	93.25±9.46	61.19±0.53	18.83±0.59	17.08±0.21	2.90±0.32
	R3	5.49±0.04	10.38±0.21	54.57±6.73	16.17±1.98	16.29±1.84	2.25±0.30	89.28±10.26	61.12±0.91	18.11±0.54	18.25±0.56	2.52±0.20
	p	*0.45*	*0.21*	*0.45*	*0.88*	*0.98*	*0.49*	*0.69*	*0.70*	*0.24*	*0.26*	*0.72*
6	R1	5.49±0.04	11.67±0.61	53.18±7.03	20.85±1.87	16.82±2.85	2.31±0.53	93.17±11.11	57.08^{ab}±1.00	22.38±1.51	18.05±0.74	2.48±0.27
	R2	5.42±0.07	11.26±0.50	55.96±5.72	18.40±2.89	16.90±2.25	2.82±0.35	94.08±10.47	59.48^{b}±0.80	19.56±0.92	17.96±0.60	3.00±0.32
	R3	5.40±0.03	11.06±0.10	58.35±7.21	18.20±2.35	17.04±1.72	2.90±0.35	96.50±10.77	60.47^{a}±0.79	18.86±0.38	17.66±0.58	3.01±0.26
	p	*0.47*	*0.64*	*0.86*	*0.31*	*0.47*	*0.57*	*0.83*	*0.03*	*0.09*	*0.13*	*0.5*
8	R1	5.47±0.04	11.41±0.26	57.39±5.56	18.42±1.94	15.49±1.98	2.71^{a}±0.13	94.01±9.11	61.05±0.41	19.59±0.62	16.48±0.5	2.88±0.18
	R2	5.55±0.10	10.66±0.30	61.76±5.97	19.09±2.64	15.67±1.92	2.60^{ab}±0.30	99.12±10.06	62.31±0.71	19.26±0.31	15.81±0.44	2.62±0.31
	R3	5.42±0.05	10.72±0.51	60.29±3.30	20.09±1.15	15.72±0.99	2.18^{b}±0.24	99.28±4.40	60.73±0.78	20.24±0.40	15.83±0.52	2.20±0.10
	p	*0.45*	*0.31*	*0.22*	*0.41*	*0.24*	*0.04*	*0.22*	*0.93*	*0.37*	*0.52*	*0.23*

NH_3: Ammonia, **AA**: acetic acid, **PA**: Propionic acid, **BA**: Butyric acid, **LA**: Lactic acid, **VFA**: volatile fatty acid, $^{a, b, c, d}$: Different letters in the same line indicate statistical difference (p 0,05)

of R2, in HCO3 of R1 and R3, in Na of R2 groups (p 0.05) between 0- and 6-hours post-feeding.

DISCUSSION

While DMI of the sheep ranged from 2421.58 to 1627.44 g/d, the OM digestion values ranged from 89.30 to 85.65% in the present study. Whereas the consumption of all nutrients decreased significantly, the digestive values tended to decrease except for starch due to the increase in starch in the diet. No difference was observed in starch digestion. In a study conducted by Sevim (16) with lambs, daily DMI (1101.87gr) and DM (46.53%) and OM (54.81%) digestion values were found to be quite low compared to the findings of the current study. Galina et al. (17) reported DMI as 1379gr/day, DMD as 79.7%, and OMD as 68.4%. The reasons for these differences are thought to be the roughage/concentrated feed ratios of the diets, the different additives added to the diet, the breed, age, and growth periods of the animals used in the experiments. As the amount of starch in the diets increased, the DMIs tended to decrease. Similar trends were reported by Alata (18), Brown et al. (19), Gozho et al. (20) and Stock (21), which supports the results of the current study. The decrease in dry matter consumption due to the increase in grains in the diet is attributed to the decrease in rumen pH, as well as meeting the energy requirements of animals more quickly compared to roughage. Especially in diets with concentrated feed, the glucose released in the rumen increases the activity of *Streptococcus bovis*, thus the amount of lactic acid, and then, causes a decrease in the rumen pH. It has also been reported that DMI decreases with a decrease in rumen pH (19, 22). DMI decreases, but the risk of acidosis increases with the consumption of concentrated feed in the diets. However, Stock (21) and Keunen et al. (23) reported that this risk is more common in diets consisting of fast-fermenting feedstuffs such as barley and wheat, compared to feedstuffs with slow fermentation properties such as corn, and the DMI decreases more sharply. Unlike acute acidosis, cattle experiencing sub-acute acidosis have not typically outward signs of illness, but often exhibit decreased feed intake and lower performance (22). Having similar findings in the current study suggested that animals have experienced sub-clinical acidosis.

It has been determined that the consumption of other nutrients of the animals was in parallel with the consumption of DM, and these nutrients were consumed most in R1 group, while they are consumed the least in R3 group. The most important reason for this is thought to be that the nutrient contents of the diets are very close to each other except starch. Although the digestion rates of the nutrients of the diets were not statistically significant, a decreasing trend has been observed in the digestion rate of nutrients, except starch, due to the increased starch level. Among the nutrients, starch has the highest average digestion rates with 92.61%, 92.33% and 92.16% for R1, R2, R3, respectively. Then, crude protein (86.37%, 86.42% and 83.53%, respectively), ADF (82.07%, 76.40% and 77.01%, respectively) and NDF (80.10%, 75.07% and 77.08%, respectively) have followed. Dietary NDF digestion rates in the current study were similar to results (77.14%) reported by Galina et al. (17). The NDF and ADF digestion rates obtained in the present study were higher than that of Fuller et al. (24; 62.7%, 35.3%, respectively), but rate of starch digestion (93.3%) was similar. The nutrient digestion rates obtained in the current study were also higher than CP, NDF and ADF digestion rates (68.72%, 68.25%, 67.77%, respectively) reported by Araujo et al. (25), and the NDF and ADF digestion rates

(40.67%, 34.73%, respectively) reported by Sevim (16). The difference between these studies was thought to be due to the differences in animal breeds used in trials, the feed ingredients used in the diet, and the differences in the ratios of roughage/concentrated feed in the diets.

Wheeler and Noller (26) found that the pH of feces was between 6.65 and 5.78 in their experiment with diets containing grains in various proportions. Luan et al. (27) noted that the feces pH of animals fed diets with various grain between 6.61 and 6.56. Apart from the similarities in the studies, the low pH is thought to be due to the differences in the amount of concentrated feed in the diets and the processing of the feedstuffs (especially

Table 4 - Blood gas values of blood samples taken from animals. (Mean ±SD).

		0. h	6. h	*p*
pH	R1	7.42±0.03	7.41±0.02	*0.72*
	R2	7.43^{A}±0.03	7.37^{B}±0.03	*0.03*
	R3	7.45^{A}±0.06	7.40^{B}±0.05	*0.04*
	*p**	*0.89*	*0.78*	
pCO_2, mmHg	R1	40.97±1.94	37.17±3.15	*0.44*
	R2	37.38^{A}±1.37	33.98^{B}±1.47	*0.04*
	R3	36.42±2.28	35.87±2.90	*0.82*
	*p**	*0.24*	*0.69*	
HCO_3, mmol/L	R1	26.38^{A}±1.73	22.37^{B}±1.02	*0.01*
	R2	24.63±1.41	21.72±1.43	*0.13*
	R3	24.03^{A}±1.92	21.33^{B}±1.08	*0.04*
	*p**	*0.61*	*0.83*	
BE, mmol/L	R1	2.33±0.68	1.88±0.70	*0.72*
	R2	2.68±0.82	1.98±0.62	*0.15*
	R3	3.13±0.40	2.87±0.49	*0.74*
	p*	0.38	0.57	
HCT, %	R1	27.00±1.34	29.50^{a}±0.67	*0.08*
	R2	25.17±1.14	26.50^{b}±0.72	*0.32*
	R3	28.5±0.81	29.17^{a}±1.14	*0.68*
	*p**	*0.14*	*0.04*	
Na, mmol/L	R1	149.55±1.25	147.90±1.35	*0.27*
	R2	147.42^{B}±0.57	149.42^{A}±0.89	*0.03*
	R3	146.58±1.93	147.27±0.62	*0.69*
	*p**	*0.32*	*0.32*	
K, mmol/L	R1	4.09±0.11	4.03±0.16	*0.78*
	R2	3.82±0.12	3.85±0.14	*0.88*
	R3	3.89±0.25	3.89±0.25	*0.99*
	*p**	*0.53*	*0.79*	
Ca, mmol/L	R1	1.03±0.04	0.99±0.03	*0.54*
	R2	0.97±0.04	1.01±0.06	*0.19*
	R3	0.95±0.08	1.03±0.05	*0.06*
	*p**	*0.62*	*0.80*	
Cl, mmol/L	R1	109.33±1.23	111.67±1.05	*0.20*
	R2	110.67±1.76	113.00±1.13	*0.08*
	R3	110.33±1.56	110.83±1.19	*0.41*
	*p**	*0.82*	*0.41*	
Lactate, mmol/L	R1	2.66±0.45	2.08±0.46	*0.48*
	R2	2.12±0.28	1.84±0.27	*0.42*
	R3	2.16±0.40	1.92±0.58	*0.39*
	*p**	*0.56*	*0.93*	

pCO2: Partial carbon dioxide, HCO3: Bicarbonate, BE: Base excess, HCT: Hematocrit, Na: Sodium, K: Potassium, Ca: Calcium, Cl: Chlorine, p: p value for rows, p*: p value for columns, a, b, c, d: Different letters in the same column show statistical difference (p 0.05), A, B: Different letters in the same row show statistical difference (p 0.05).

Biochemistry parameters of blood samples taken from animals are given in Table 5. Among the biochemistry parameters of the blood samples taken from the animals, it was determined that only creatine of R1 group was statistically significant between 0- and 6-hour post-feeding (p 0.05), while other values were not statistically significant (p>0.05).

barley).

Li et al. (28) also reported that the pH of the feces of animals with acidosis decreased. In the present study, the decrease in feces pH parallel to the increase in the amount of starch in the diet supports the findings of Li et al. (28).

Among the sampling hours, the lowest pH was detected in R3 group at 6 hours (5.40), and the highest pH was observed in R2 group at 0 hours (6.00) post-feeing. VFA and lactate, which are formed as a result of the fermentation of carbohydrates in the anaerobic rumen environment, cause a decrease in rumen pH. This situation becomes more evident in cases where a lot of starch is consumed (22). Therefore, all ration groups in the study are similar to the aforementioned studies and are within the limits of acidosis. Rumen pH below 5.8 according to Beauchemin (29), and Nordlund and Garrett (30), below 5.6 according to Cooper et al. (31) has been considered as subacute rumen acidosis. It has also been reported that a ruminal pH of 5.6 has been considered as a benchmark of subacute acidosis (22). Based on literature values mentioned above, the pH values obtained in this study are thought to indicate typical subacute acidosis.

The lowest rumen ammonia-N level was 8.49 mmol/L in the study. It is seen that this value is above the level of 5 mg ammonia-N/dL (32), which is required for optimum in vivo microbial protein synthesis. The ammonia- N values obtained in the current study were similar to those reported by Alata (18) as 9.12 and 12.59 mmol/L from animals with subacute acidosis, and the ammonia-N levels determined by Gülmez (33) with various carbohydrate sources (11.10-12.50 mmol/L). L). Ammonia, which is specially produced by the decomposition of non-protein nitrogen (NPN) is a substance used in microbial protein synthesis. It also plays a role in buffering the rumen against acidosis (22). As a result of the increase in the amount of concentrated feed in the diet, the number of cellulotic bacteria decreases in the decreasing pH of the rumen. The decrease in the number of cellulotic bacteria using ammonia with the decrease in pH causes an increase in ammonia-N level (34, 35).

Although there was no statistical difference in the amount of VFA between the diets, relative changes were observed depending on the microbial activity. The amounts of AA, PA, BA and LA in this study were similar to the values determined by Alata (18; 53.97, 22.90, 17.38 and 0.86 mmol/L, respectively) and Alta (36; 56.92, 19.07, 17.37 mmol/L, respectively). Morgan et al. (37) reported AA, PA, BA and LA values as 91.33, 38.94, 15.35 and 2.89 mmol/L, respectively. When these results were compared with the results of the current study, while the amounts of AA and PA were lower in the current study, the amounts of BA and LA were similar to this study. Although Krause and Combs (38) increased the rate of rapidly digestible starch in the diet, an increase in the amount of VFA was not observed.

McLeod and Baldwin (39) stated that if the concentrated feed

Table 5 - Biochemistry parameters of blood samples taken from animals. (Mean ±SD).

		0. h	6. h	*p*
GLU. mg/dl	R1	85.83±3.56	87.33±2.47	*0.70*
	R2	85.17±2.82	89.17±1.78	*0.21*
	R3	88.50±1.77	90.00±2.28	*0.16*
	*p**	*0.62*	*0.42*	
TP. g/dl	R1	7.79±0.14	7.79±0.18	*0.98*
	R2	7.52±0.10	7.57±0.15	*0.62*
	R3	7.60±0.11	7.64±0.12	*0.76*
	*p**	*0.30*	*0.60*	
TG. mg/dl	R1	25.17±2.82	24.17±2.43	*0.72*
	R2	16.50±1.98	21.00±2.97	*0.15*
	R3	21.17±2.36	21.33±2.16	*0.95*
	*p**	*0.07*	*0.63*	
Urea. mg/dl	R1	41.64±3.96	43.66±2.17	*0.54*
	R2	39.13±3.97	38.94±4.33	*0.95*
	R3	39.08±5.70	36.11±4.22	*0.22*
	*p**	*0.91*	*0.37*	
CREA. mg/dl	R1	0.69^{A}±0.03	0.63^{B}±0.03	*0.01*
	R2	0.66±0.03	0.65±0.03	*0.47*
	R3	0.67±0.02	0.65±0.03	*0.47*
	*p**	*0.73*	*0.90*	
ALB. g/dl	R1	2.35±0.08	2.38±0.05	*0.47*
	R2	2.38±0.06	2.37±0.09	*0.77*
	R3	2.45±0.05	2.43±0.05	*0.74*
	*p**	*0.54*	*0.76*	

GLU: Glucose, TP: Total protein, TG: Triglyceride, CREA: Creatinine, ALB: Albumin, p: P value for rows, p*: P value for columns, a, b, c, d: Statistical difference of different letters in the same column (p 0.05), A, B: Different letters on the same line indicate statistical difference (p 0.05).

ratios of the diets increase, the ion passages in the rumen wall increase and the absorption of VFA increases and thus, the increase in the amount of VFA in the rumen is tried to be prevented. Increasing the ratio of concentrated feeds in the rations causes a decrease in rumen pH as a result of which bacteria such as *Streptococcus bovis* increase lactic acid production. Bacteria such as *Ruminobacter amylophilus and Selenomonas ruminantium* consume the produced lactic acid and act as a kind of buffer. However, if the pH drops below 5.5, the chance of survival of these bacteria decreases and their death increases as acidosis becomes severe. Therefore, lactic acid and VFA accumulation increases in the rumen (40; 41).

Blood pH, pCO_2, HCO_3 and BE values decreased at 6 hours post-feeding. The decrease in these values are the causes and consequences of acidosis. The low levels of HCO_3 and BE indicate the severity of acidosis and efforts to compensate for it. At the same time, high VFA in the rumen decreases their rate in the blood (42).

The pH (7.45, 7.46) and pCO_2 (35.44, 36.08 mmHg) values in the study of Alata (2013) were similar to the values obtained in the current study. Among other blood gas parameter, K (3.54, 3.68 mmol), Ca (0.87, 0.85 mmol), lactate (0.63, 0.53 mmol), hematocrit (25.33%, 25.55%) and BE (1.81, 1.26 mmol/L) values were lower while the Na (154, 156 mmol) and HCO_3 (25.53, 25.21 mmol) values were higher compered to the current study. The results obtained by Polat (2018) from animals with latent acidotic stress, while pH (7.41) value was similar, pCO_2 (46.98 mmHg), HCO_3 (30.85 mmol/L) and (BE 7.35 mmol/L) values were higher compared to the current study. Morgante et al. (43) also reported results similar to those of Polat (44).

Differences between these values were associated with the severity of acidosis in animals, breed and health status of the animal. In intensive concentrate feed consumptions, the pH of the blood as well as the pCO_2 and HCO_3 values decrease. Low pCO2 and HCO3 values with pH in blood gas values are indicative of acidosis (metabolic). This situation occurs as a result of the intense production of acid in the rumen and the slowing of absorption. In this case, it increases the absorption of cations such as Na and K in order to reduce rumen osmolality (22, 45). Based on Kayar (46), reference values for blood biochemistry values in cattle are as follow; glucose 40-60 mg/dl (sheep 40-60 mg/dl), total protein 6.7-7.5 g/dl (sheep 6.0-7.9 g/dl), triglyceride 15.45 mg/dl, urea 20-40 mg/dl (sheep 20-30 mg/dl), creatinine 1-2 mg/dl (sheep 0.6-1.5 mg/dl), albumin 3.0-3.6 g/dl (2.4-3.9 g/dl). According to the reference values, all values except creatinine and albumin in this study were high. im ek and Sekin (47) found TP, urea and creatinine to be 6.85-6.78 mg/dl, 23.42-23.62 mg/dl, 1.38-1.37 mg/dl at 0 and 6 hours post-feeding, respectively, in sheep with acidosis. When the results of this study were compared with the results of the current study, creatinine was higher, while total protein and urea were lower. On the other hand, Alkabi et al. (48) reported taht total protein, albumin, glucose, urea and creatinine were 8.18 g/dl, 3.73 g/dl, 64.36 mg/dl, 42.35 mg/dl, 1.23 mg/dl, respectively, in sheep with acidosis, comparing with the results of the study, glucose was lower, total protein, albumin, urea and creatinine were higher. It has been reported that high urea and creatinine levels may cause deterioration in kidney functions, and high total protein and albumin may be due to losses due to fluid passing from the blood to the digestive system as a result of dehydration in acidosis (49, 50).

CONCLUSION

It can be concluded that increasing the starch level above 25% in the diets of lambs adversely affects the nutrient consumption and nutrient digestion in general, and also reduces the blood pH and HCO_3 levels, thus increasing the risk of subacute acidosis.

Ethical Approval: This study was deemed appropriate by the meeting of the Kırıkkale University Ethic Committee dated 06.11.2019 and the decision numbered 2019-12/55.

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