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Diurnal variations of renal activity in goats

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Abstract: Research was conducted on 12 clinically healthy 4-year-old White Nobel goats. Renal activity examinations were carried out with the use of clearance methods where inulin was used as a testing substance to determine the level of glomerular filtration. The results allowed determination of inulin clearance, endogenic creatinine, sodium, potassium, and chloride levels. Filtered load, tubular resorption, and excretion in urine of sodium, potassium, and chlorides were marked. The research revealed diurnal variations of renal activity in goats. Most of the rhythms' acrophases occur in the phase of activeness between 1200 and 1600 hours. The resorption rhythm of potassium in the renal tubule differs from glomerular filtration by 12 h and its acrophases occur in the resting period. The main reasons for diurnal variations of the electrolyte excretion by kidneys are variations of the glomerular filtration rate. Feeding time does not affect the diurnal variations of renal activity. Due to the circadian glomerular filtration variations, resorption and secretion processes in nephrons, and, in consequence, the changes of the final urine composition, the kidneys lead to the organism's adaptation to changes in the environment and also in the circadian rhythm.

Key words: Goats, diurnal variations, renal activity, glomerular filtration rate

1. Introduction

Biological rhythms are one of the examples that living organisms have adapted to the environment. Of all biological rhythms, diurnal variations are of most interest as a sign of living creatures' adaptation to the environment and a factor increasing the chance of maintaining homeostasis (1). Studies on chronobiological aspects of physiological functions have been carried out mainly on humans and laboratory animals. A few studies have been carried out on livestock (2–4).

Kidneys are the main organ regulating the internal environment. They are responsible for maintaining the total volume of water and its distribution in particular water spaces, for electrolyte composition of bodily fluids and especially extracellular fluid, and for maintaining acid-base balance. Moreover, kidneys are responsible for expelling metabolites from the organism, especially for protein metabolism and other unnecessary and harmful substances, and at the same time maintaining the necessary substances in blood (4). Kidneys take part in the synthesis of hormones or their precursors such as renin, renal erythropoietic factor, calcitriol, and prostaglandins (5–7).

Renal activity in farm animals has been studied for many years. The results led to the recognition of the processes occurring in kidneys in reference to the regulation of the water-electrolyte system in animals (6–8). There are no available studies assessing the renal activity in farm animals in terms of the chronobiological aspect. Studies on renal activity diurnal variation have been carried out mainly on humans (9,10) and laboratory animals (11). A few studies have been carried out on livestock. Such research carried out on 4-week-old goats was done by Vogel (12), but he considered only the renal activity variations between "daytime", 0800 hours, and "nighttime", 2000 hours. The aim of this study was to examine circadian renal activity in goats in terms of water-electrolyte homeostasis regulation and the analysis of the circadian rhythms if such should appear.

2. Materials and methods

The research was carried out on 12 clinically healthy 4-year-old White Nobel goats. The animals were fed twice a day at 0700 and 1600 hours. The feed ration consisted of 0.7 kg of concentrated feedstuff (containing 16%–18% of protein), 0.4 kg of dry beet pulp, hay, and water (ad libitum). Ten days ahead of the research and during the research a strict light regime was kept of LD 12:12 (12 h of light from 0630 to 1830 hours and 12 h of darkness from 1830 to 0630 hours).

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Before the research a polyethylene drain was applied to the external jugular vein, which allowed for fast and stress-free blood sampling. The goats were also catheterized with a balloon catheter.

Renal activity examinations were carried out with the use of clearance methods, where inulin was used as a testing substance to determine the level of glomerular filtration. In the research, due to the relatively fast renal excretion of inulin after a single injection, we applied a unique injection method. Inulin was implanted subcutaneously in the form of retard tablets in order to maintain a stable concentration of inulin in blood plasma for 48 h. Their usefulness in clearance examinations was a subject of previous research where the authors analyzed the kinetics of testing substance release and its concentration in blood and urine (13).

Testing began at 0830 hours with collecting blood and urine samples ("0" samples). Next the inulin retard tablets were implanted subcutaneously on the neck. The tablets were prepared in the Department of Pharmaceutical Chemistry of Graz University (Austria) according to standard procedure. Eudragit (Rohm Pharma GmbH) was used as an inulin carrier and was mixed with inulin in the ratio of 2.5:3. The obtained plastic mass was dried in a vacuum dryer (1 bar pressure, temperature 20 °C) within 24 h and granulated (the size of the pellets was 200–315 µm). Pressure of 10 T was applied to mold the pellets. Ready tablets were 5.5 g in weight, 3 cm in diameter, and 0.6 cm in height. Directly after the implantation, the goats were intravenously given an initial dose of inulin in the amount of 40 mg/kg of body weight in order to saturate the distribution space.

Clearance (C) examinations were carried out 3 h after the initial dose of inulin. Urine was collected (within 20 min) in 3-h intervals over 48 h. The blood was sampled into test tubes with heparin (250 i.m., Heparinum, Polfa) 30 min, 1 h, and 2 h after the initial inulin dose and in the middle of every urine collection period. The blood was centrifuged and the plasma, together with the urine samples, was cooled down to 4 °C. In the urine and plasma samples, the inulin concentration was marked with the use of the resorcinol method (14), endogenous creatinine concentration with the use of the Folin–Wu method, sodium and potassium concentrations with the use of the flame emission spectrometry method (Flapho-4), and chloride concentration with the use of the potentiometric titration method (Spexon chlorimeter). The results allowed calculating inulin clearances (C_{in}), endogenous creatinine (C_{cr}), sodium (C_{Na}), potassium (C_K), and chlorides (C_{Cl}). Filtered load (F), tubular resorption (TR), and excretion in urine (UV) of sodium, potassium, and chlorides were also calculated.

Renal activity indicators were calculated according to the following formulas:

$C = UV / P$,

$F = P \times GFR$,

$UV = U \times V$,

$TR = (F - UV / F) \times 100\%$,

where C is clearance (mL/min), UV is excretion in urine (µmol min^{-1} m^{-2}), P is plasma level (mmol/L), F is filtered load (mmol/min), GFR is glomerular filtration rate (mL min^{-1} m^{-2}), U is substance concentration in urine (mmol/L), V is diuresis (mL/min), TR is tubular resorption (%), and F is filtered load (mmol/min).

The results were standardized per 1 m^2 of goats' body surface area according to the formula $S = 0.105 \sqrt[3]{\text{m.c.}}$, where S is body surface area (m^2) and m.c. is body weight (kg).

The results were then subjected to statistical analysis with the use of one-way analysis of variance and Duncan's multiple range test (Statistica v. 6.0). In order to confirm the diurnal rhythms and determine the rhythm period and its acrophase, all the time series were analyzed with the use of Chronos v. 1.0. (15). The results are presented in Tables 1–4.

3. Results

Inulin concentration and the values of the glomerular filtration measured with the levels of inulin clearances and endogenous creatinine are presented in Table 1. In the first 3 h of the experiment, inulin excretion from the blood was relatively fast and its concentration decreased from 4.02 mg% to 3.09 mg%. From the 6th to the 48th hour, due to gradual inulin release from the implanted tablets, its concentration in plasma was relatively stable and remained at the level of 2.46 mg% to 0.59 mg%. During the experiment inulin was eliminated from the urine with an average speed of 0.06 mg/h. C_{in} level was used as an estimate for the GFR. C_{in} levels were highest in the afternoon of day 1 at 1200 hours (62.05 mL min^{-1} m^{-2}) and day 2 at 1500 hours (59.72 mL min^{-1} m^{-2}). The lowest levels of C_{in} were observed at night (at 0300 hours) of the first and second day of the experiment at 46.43 mL min^{-1} m^{-2} and 47.15 mL min^{-1} m^{-2}, respectively. The differences were statistically significant at $P \leq 0.01$. The GFR rhythm period was 25.88 h with the acrophase at 1340 hours. Circadian changes of day-night C_{cr} levels were also statistically significant ($P \leq 0.01$). The highest C_{cr} levels were observed during the day at 1500 hours (day 1 – 67.74 mL min^{-1} m^{-2}, day 2 – 68.8 mL min^{-1} m^{-2}), and the lowest values were respectively seen at night, at 0600 hours the first night (60.00 mL min^{-1} m^{-2}) and at 0300 hours the second night (61.34 mL min^{-1} m^{-2}). The C_{cr} rhythm period was 22.76 h with acrophase at 1501 hours. Significant differences between C_{in} and C_{cr} were found in all the clearance periods. C_{cr} levels were

Table 1. Mean values of inulin plasma concentration (P_{in}), inulin clearance (C_{in}), endogenous creatinine (C_{cr}), and the periods and acrophases of rhythms in goats over the studied time span.

		Hour																Rhythm period	Rhythm acrophase
		1200 A	1500 B	1800 C	2100 D	0000 E	0300 F	0600 G	0900 H	1200 I	1500 J	1800 K	2100 L	0000 M	0300 N	0600 P	0900 R	Hours	Hour
P_{in} (mg%)	$\overline{X}$	4.02	3.09	2.46	2.35	2.32	2.16	1.98	1.81	1.61	1.50	1.32	1.17	0.94	0.78	0.69	0.59	-	-
	± SD	1.55	083	0.64	0.52	0.47	0.42	0.34	0.29	0.28	0.26	0.24	0.29	0.21	0.21	0.20	0.18		
C_{in} (mL min^{-1} m^{-2})	$\overline{X}$	62.05	58.61	55.75	51.62	47.98	46.43	47.28	52.77	57.74	59.72	57.47	57.76	49.83	47.15	49.88	52.95	25.88	1340
	± SD	4.20	4.32	4.48	5.60	6.30	5.14	6.11	4.38	3.10	2.45	3.60	4.10	4.80	5.86	5.24	3.96		
C_{cr} (mL min^{-1} m^{-2})	$\overline{X}$	67.73	67.74	65.90	63.83	61.02	60.36	60.00	64.92	68.73	68.81	65.47	62.61	61.50	61.34	63.16	67.48	22.76	1501
	± SD	8.26	7.66	6.86	7.32	6.96	7.14	7.56	7.01	7.73	8.60	7.28	6.33	5.71	6.94	7.26	7.07		

Significant differences ($P \leq 0.01$): C_{in} A→DEFG, B→EFG, J→MNP, K→MNP; C_{cr} A→EFG, B→EFG, I→MNP, J→MNP.
Explanations: A→DEFG – significant differences between C_{in} at 1200 hours (A) and C_{in} at 2100 (D), 0000 (E), 0300 (F), and 0600 (G) hours.

8.4%–30% (4.85–14.53 mL min^{-1} m^{-2}) higher compared to C_{in} levels. The biggest differences between the clearances of both substances were found at night and early in the morning.

Sodium concentration in plasma remained relatively stable, fluctuating between 136.92 mmol/L and 139.42 mmol/L (Table 2) through the whole research period. F_{Na} in the renal glomerulus was stable and changed proportionally to the changes of GFR, so its circadian rhythm was statistically significant (Table 2). The highest F_{Na} values were observed in the afternoon at 1200 hours on day 1 and at 1500 hours on day 2 (8.50 mmol min^{-1} m^{-2} and 8.32 mmol min^{-1} m^{-2}, respectively). The lowest values of the filtered load of sodium were observed at 0300 hours (6.39 mmol min^{-1} m^{-2} the first night and 6.47 mmol min^{-1} m^{-2} the second night). Rhythm period was 25.07 h with the acrophase at 1356 hours. TR_{Na} was high throughout the research period and it ranged from 99.44% to 99.83% compared to the filtered load (Table 2). Absorption of this electrolyte was the highest at 0900 hours on day 1 and between 0000 and 0300 hours on day 2 and it reached the levels of 99.72% and 99.83%, respectively. The lowest values of TR_{Na} of 99.44% were observed during the day at 1200 hours on both the first and the second day. The observed differences were statistically insignificant. Rhythm period was 26.48 h long with the acrophase at 2153 hours. Circadian changes of urinary excretion of sodium (UV_{Na}) were found (Table 2). The highest levels of UV_{Na} were observed at 1200 hours during the first 2 days (0.048 mmol min^{-1} m^{-2} and 0.045 mmol min^{-1} m^{-2}, respectively). The lowest values of UV_{Na} occurred at night at 0000 hours the first night (0.019 mmol min^{-1} m^{-2}) and 0300 hours the second night (0.011 mmol min^{-1} m^{-2}). The changes were statistically confirmed at $P \leq 0.01$. The rhythm period was 25.67 h long with the acrophase at 1220 hours. C_{Na} also showed circadian variations (Table 2). The highest values of C_{Na} were observed at 1200 hours on days 1 and 2 of the experiment and were respectively at the level of 0.35 mL min^{-1} m^{-2} and 0.3 mL min^{-1} m^{-2}. The lowest values occurred on the first night between 2100 and 0000 hours (0.14 mL min^{-1} m^{-2}) and the second night at 0300 hours (0.08 mL min^{-1} m^{-2}). The observed differences between the minimal and maximal C_{Na} levels were statistically significant at $P \leq 0.01$. Rhythm period was 25.74 h with acrophase at 1216 hours.

Potassium concentration in blood plasma remained at the level of 3.92–4.08 mmol/L (Table 3). Rhythm period was 23.18 h with acrophase at 1545 hours. During the experiment, F_K changes were parallel to F_{Na} and were proportional to C_{in} changes (Table 3). The highest values of F_K were observed in the first 24 h of the experiment at 1200 hours (0.25 mmol min^{-1} m^{-2}) and in the second 24 h at 1500 hours (0.24 mmol min^{-1} m^{-2}). The lowest F_K values occurred both on the first and second day at 0300 hours (0.18 mmol min^{-1} m^{-2} and 0.19 mmol min^{-1} m^{-2}). The differences between the observed values were statistically significant at $P \leq 0.01$. Rhythm period was 25.18 h with acrophase at 1349 hours. TR_K showed a diurnal rhythm (Table 3). The highest levels were observed at 0300 hours and they reached the value of 82.92% on the first day and 82.16% on the second day. The lowest values of TR_K were observed at 1200 hours (77.07%) on the first day and at 1500 hours (75.94%) on the second day. The observed differences were statistically insignificant. Rhythm period was 23.44 h with acrophase at 0204 hours. Diurnal changes of UV_K (Table 3) were observed. Statistically significant ($P \leq 0.01$) differences of diurnal urinary excretion of potassium were also noted. Maximal excretion appeared at 1200 hours the first day and at 1500 hours the second, whereby UV_K was respectively at the levels of 56.99 µmol min^{-1} m^{-2} and 58.41 µmol min^{-1} m^{-2}. The lowest renal excretion of K was observed at 0300 hours; UV_K was respectively at the level of 30.46 µmol min^{-1} m^{-2} the first night and 33.44 µmol min^{-1} m^{-2} the second night. Rhythm period was 24.23 h with acrophase at 1401 hours. F_K, TR_K, and UV_K changes resulted in C_K changes (Table 3). The changes were statistically significant at $P \leq 0.01$. The highest values of C_K of 14.24 mL min^{-1} m^{-2} the first day at 1200 hours and 14.40 mL min^{-1} m^{-2} the second day at 1500 hours were reported. The lowest levels of C_K were found at 0300 hours and their values were respectively 7.86 mL min^{-1} m^{-2} on the first day and 8.38 mL min^{-1} m^{-2} on the second day. The rhythm period was 24 h and 17 min with acrophase at 1411 hours.

Chloride concentrations in goats' plasma ranged between 102.08 and 106.17 mmol/L (Table 4) and was statistically insignificant. The highest F_{Cl} levels were found at 1200 hours on the first day (6.40 mmol min^{-1} m^{-2}) and at 1500 hours on the second day (6.33 mmol min^{-1} m^{-2}). The lowest values of F_{Cl} were observed at 0300 hours the first night (4.72 mmol min^{-1} m^{-2}) and 0600 hours the second night (5.11 mmol min^{-1} m^{-2}). F_{Cl} changes showed diurnal rhythm and the differences between the highest and the lowest values were significant at $P \leq 0.01$. Rhythm period was 25.77 h with acrophase at 1257 hours. TR_{Cl} was stable during the experiment and ranged between 97.21% and 98.66% compared to filtration rate (Table 4). The observed differences were statistically insignificant. The lowest levels of TR_{Cl} were observed in the mornings of days 1 and 2 at 0900 hours (97.21% and 97.88%, respectively). UV_{Cl} levels showed clear diurnal variations (Table 4). The differences were significant at $P \leq 0.01$. The highest chloride excretion occurred during the day at 1500 hours on the first day at 0.126 mmol min^{-1} m^{-2} and at 1200 hours on the second day at 0.129 mmol min^{-1} m^{-2}. The lowest levels of UV_{Cl} were observed on the first two days at night at 0300 hours (0.103

Table 2. Mean values of sodium plasma concentration (P), filtered load (F), tubular reabsorption (TR), urinary volume (UV), and clearance (C) and the periods and acrophases of rhythms.

		Hour																Rhythm period	Rhythm acrophase
		1200 A	1500 B	1800 C	2100 D	0000 E	0300 F	0600 G	0900 H	1200 I	1500 J	1800 K	2100 L	0000 M	0300 N	0600 P	0900 R	Hours	Hour
P	$\overline{X}$	137.00	136.92	137.42	137.92	137.33	137.67	138.08	137.92	138.00	139.42	138.08	138.50	138.25	137.17	138.67	137.67	57.66	1542
(mmol/L)	± SD	3.22	3.53	3.99	4.14	4.33	3.72	3.12	4.46	4.45	3.55	3.29	4.46	4.22	3.24	4.27	4.92		
F	$\overline{X}$	8.50	8.02	7.57	7.11	6.58	6.39	6.48	7.28	7.98	8.32	7.94	9.46	6.80	6.47	6.93	7.30	25.07	1356
(mmol min^{-1} m^{-2})	± SD	0.63	0.62	0.70	0.79	0.85	0.74	0.79	0.70	0.51	0.43	0.51	0.69	0.88	0.82	0.81	0.30		
TR	$\overline{X}$	99.44	99.54	99.66	99.72	99.70	99.57	99.56	99.52	99.44	99.64	99.66	99.82	99.83	99.83	99.67	99.48	26.48	2153
(%)	± SD	0.06	0.26	0.24	0.15	0.05	0.30	0.28	0.39	0.10	0.25	0.33	0.13	0.12	0.11	0.17	0.33		
UV	$\overline{X}$	0.048	0.037	0.026	0.020	0.019	0.027	0.027	0.029	0.045	0.030	0.026	0.015	0.012	0.011	0.023	0.038	25.67	1220
(mmol min^{-1} m^{-2})	± SD	0.008	0.021	0.018	0.012	0.004	0.017	0.016	0.023	0.009	0.022	0.025	0.013	0.009	0.008	0.012	0.024		
C	$\overline{X}$	0.35	0.27	0.19	0.14	0.14	0.20	0.20	0.21	0.33	0.22	0.19	0.11	0.09	0.08	0.016	0.27	25.74	1216
(mL min^{-1} m^{-2})	± SD	0.05	0.15	0.14	0.08	0.03	0.12	0.11	0.17	0.06	0.16	0.18	0.09	0.06	0.06	0.09	0.17		

Significant differences ($P \leq 0.01$): F_{Na} A→DEFG, B→EFG, I→MNP, J→MNP; UV_{Na} A→CDE, B→DE, H→LMNP, I→KLMNP; C_{Na} A→CDEFGH, B→DE, H→LMNP, I→KLMNP.

Explanations: A→DEFG – significant differences between F_{Na} at 1200 hours (A) and F_{Na} at 2100 (D), 0000 (E), 0300 (F), and 0600 (G) hours.

Table 3. Mean values of potassium plasma concentration (P), filtered load (F), tubular reabsorption (TR), urinary volume (UV), and clearance (C) and the periods and acrophases of rhythms.

		Hour																Rhythm period	Rhythm acrophase
		1200 A	1500 B	1800 C	2100 D	0000 E	0300 F	0600 G	0900 H	1200 I	1500 J	1800 K	2100 L	0000 M	0300 N	0600 P	0900 R	Hours	Hour
P	$\overline{X}$	3.94	4.08	4.04	3.93	3.94	3.92	3.92	4.03	4.05	4.05	4.06	4.02	3.96	3.98	3.98	4.03	23.18	1545
(mmol/L)	± SD	0.35	0.34	0.31	0.30	0.38	0.45	0.35	0.30	0.29	0.34	0.27	0.24	0.22	0.29	0.25	0.33		
F	$\overline{X}$	0.25	0.24	0.23	0.20	0.19	0.18	0.19	0.21	0.23	0.24	0.23	0.22	0.20	0.19	0.20	0.22	25.18	1349
(mmol min^{-1} m^{-2})	± SD	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.02	0.02	0.03	0.04	0.03	0.04		
TR	$\overline{X}$	77.07	79.16	78.87	80.10	81.64	82.92	80.69	78.27	76.14	75.94	77.39	79.49	81.99	82.16	79.68	76.53	23.44	0204
(%)	± SD	6.46	6.02	5.00	5.49	5.53	4.64	4.35	3.78	4.00	3.81	3.44	1.77	2.69	2.57	1.71	4.20		
UV	$\overline{X}$	56.99	49.73	47.93	40.38	34.98	30.46	35.92	46.50	55.65	58.41	52.08	44.35	35.68	33.44	40.29	52.05	24.23	1401
(mmol min^{-1} m^{-2})	± SD	18.83	15.70	13.15	12.40	12.94	9.68	10.59	11.36	10.84	10.26	6.31	5.83	7.18	7.91	7.95	16.48		
C	$\overline{X}$	14.24	12.28	11.86	10.30	8.90	7.86	9.13	11.52	13.72	14.40	12.90	11.03	8.99	8.38	10.08	12.84	24.17	1411
(mL min^{-1} m^{-2})	± SD	4.22	3.96	3.12	3.15	3.12	2.23	2.44	2.42	2.23	2.15	1.90	1.14	1.59	1.71	1.51	3.67		

Significant differences ($P \leq 0.01$): F_K A→EFG, B→EFG, J→MNP, K→N; UV_K A→CDEFG, B→EFG, I→MNP, J→LMNP; C_K A→EFG, B→EFG, I→MN, J→MNP.

Explanations: A→EFG – significant differences between F_K at 1200 (A) hours and F_K at 0000 (E), 0300 (F), and 0600 (G) hours.

Table 4. Mean values of chloride plasma concentrations (P), filtered load (F), tubular reabsorption (TR), urinary volume (UV), and clearance (C) and the periods and acrophases of rhythms.

		Hour																Rhythm period	Rhythm acrophase
		1200 A	1500 B	1800 C	2100 D	0000 E	0300 F	0600 G	0900 H	1200 I	1500 J	1800 K	2100 L	0000 M	0300 N	0600 P	0900 R	Hours	Hour
P	$\overline{X}$	103.25	102.08	102.08	103.67	102.75	102.47	106.08	103.08	104.87	106.00	104.83	105.67	106.17	103.92	102.50	104.83	126.22	1837
(mmol/L)	± SD	4.71	4.91	4.29	5.68	4.09	5.87	3.82	4.70	5.27	4.33	4.43	6.08	4.47	3.09	5.85	4.04		
F	$\overline{X}$	6.40	5.98	5.68	5.33	4.92	4.75	4.99	5.48	5.91	6.33	6.02	5.68	5.29	5.15	5.11	5.54	25.77	1257
(mmol min^{-1} m^{-2})	± SD	0.51	0.45	0.39	0.44	0.61	0.54	0.64	0.50	0.53	0.30	0.37	0.54	0.55	0.74	0.59	0.35		
TR	$\overline{X}$	98.13	97.87	97.80	97.86	97.74	97.70	97.87	97.21	97.90	98.30	98.59	98.52	98.44	98.66	98.33	97.88	43.26	329
(%)	± SD	1.09	1.32	1.25	1.51	1.11	1.54	1.40	1.97	1.37	1.04	0.77	0.95	1.18	0.55	1.09	0.86		
UV	$\overline{X}$	0.122	0.126	0.125	0.115	0.110	0.103	0.106	0.125	0.129	0.109	0.086	0.087	0.088	0.068	0.087	0.119	22.52	1539
(mmol min^{-1} m^{-2})	± SD	0.079	0.079	0.073	0.082	0.055	0.066	0.069	0.108	0.087	0.068	0.050	0.062	0.073	0.030	0.057	0.052		
C	$\overline{X}$	1.09	1.24	1.23	1.14	1.04	1.03	0.99	1.23	1.23	1.02	0.88	0.82	0.82	0.65	0.84	1.14	21.88	1635
(mL min^{-1} m^{-2})	± SD	0.74	0.79	0.73	0.90	0.47	0.62	0.63	1.10	0.83	0.64	0.40	0.55	0.64	0.29	0.54	0.52		

Significant differences ($P \leq 0.01$): F_{Cl} A→EFG, B→FG, J→NP, K→P; UV_{Cl} B→EF, C→EF, I→KLMNP, N→R; C_{Cl} B→EFG, C→EFG, I→KLMNP, N→R.

Explanations: A→EFG – significant differences between F_{Cl} at 1200 hours (A) and F_{Cl} at 0000 (E), 0300 (F), and 0600 hours (G).

mmol min^{-1} m^{-2} and 0.068 mmol min^{-1} m^{-2}, respectively). Rhythm period was 22.52 h with acrophase at 1539 hours. C_{Cl} levels ranged between 0.65 mL min^{-1} m^{-2} and 1.24 mL min^{-1} m^{-2} throughout the research (Table 4). The highest C_{Cl} values were observed during the day, at 1500 hours on the first day (1.24 mL min^{-1} m^{-2}) and between 0900 and 1200 hours on the second day (0.123 mL min^{-1} m^{-2}). The lowest values of C_{Cl} were found the first night at 0600 hours (0.99 mL min^{-1} m^{-2}) and the second night at 0300 hours (0.65 mL min^{-1} m^{-2}). The differences were statistically confirmed at $P \leq 0.01$. Rhythm period was 21.88 h with acrophase at 1636 hours.

4. Discussion

Renal activity in goats shows diurnal activity variations. Acrophases of most rhythms appear in the active phase of the animal from 1200 to 1600 hours. GFR diurnal rhythm in the renal glomerulus in goats occurred with the use of inulin as well as with endogenous creatinine. The highest GFR levels measured with inulin clearances were found during the day and the lowest at night. Similar tendency of GFR variations measured with inulin clearance in 4-week-old calves was found by Skrzypczak et al. (16) (C_{in} 1000 hours = 60.39 mL min^{-1} m^{-2}; C_{in} 2200 hours = 49.99 mL min^{-1} m^{-2}). Our results confirm the results of the research conducted by other authors on humans (17–19). Stribrna and Brodan (20) claimed that high GFR levels in the afternoon occur due to higher blood pressure, which may lead to the increase of the GFR. In the presented research, the highest C_{cr} levels were also found during the daytime; however, there was 1 h of difference in acrophases. The results correspond to the results of the research conducted on humans by Buzio et al. (21). Koopman et al. (17) found the circadian rhythm of C_{cr} in humans, but only in 60%–70% of the analyzed group. Similar results were obtained by Van Acker et al. (8), who found circadian rhythm of C_{cr} in 65% of the analyzed population. In the group similar C_{in} and C_{cr} daily rhythms and similar acrophases were found.

In the conducted experiment, it was found that C_{cr} levels were higher than C_{in} levels throughout the day. Bigger differences were found during the night. This confirms the data on tubular secretion of creatinine and shows that its clearance cannot always be an objective measure of glomerular filtration. Despite the differences in the level of both clearances, their rhythms and phases coincided. Similar results were found by Koopman et al. (2), who proved coinciding GFR rhythms' phases measured with the level of C_{cr} and C_{in}. Our results, as well as the results of Koopman et al. (2) and Van Acker et al. (8), show that tubular secretion of endogenic creatinine (measured with creatinine and inulin clearance rates) is the highest at the time of bathyphase of GFR rhythm (C_{in}) and the lowest at the time corresponding to the acrophase of the rhythm. Different results were found by Sirota et al. (22). They found that C_{in} and C_{cr} levels did not show any circadian differences and the C_{cr} levels were lower than C_{in} levels ($C_{cr}/C_{in} = 0.95$).

The results of many experiments showed that the feeding time and the diet (especially the amount of protein) modify renal activity (17–21). It is known that the protein intake leads to renal hypoperfusion and plasma hyperfiltration in renal glomerulus. Among others, Luke et al. (23) found convergence of GFR levels' diurnal changes and the feeding time in rats. The authors also found a lack of GFR rhythms in a rat population with permanent food access.

In our research, a modifying effect of food (feeding time) on diurnal renal activity changes was not found. This can be confirmed by the levels of C_{in} and C_{cr} in the afternoon hours, when the levels were lowered despite the feeding time at 1600 hours. The results are not, however, inconsistent with the literature, as they do not eliminate the possibility of renal plasma filtration increase in the examined animals after feeding.

The relation of F_{Na} and UV_{Na} levels from GFR circadian variations found in the experiment is the result of the stable level of this electrolyte in blood plasma. At the same time, it is related to its constant resorption in renal tubules. That is why C_{Na} shows a circadian rhythm at the time and acrophase similar to GFR rhythms. Similar relations between the filtered load concentrated in plasma, resorbed and excreted with urine, and the GFR level appear for chlorides, which, apart from sodium, are the most osmotically active electrolyte of the extracellular fluid. For diurnal variations of potassium concentration in blood plasma, as well as the variable that shows circadian rhythm, its resorption in renal tubule modifies the influence of glomerular filtration on excreting the electrolyte from plasma. It is worth paying attention to the relation between potassium excretion from the organism and the amount of the electrolyte reaching the tubules. The rhythm's acrophase of potassium resorption in the renal tubule corresponds to the rhythm's bathyphase of glomerular filtration. With a decreasing level of filtered load, the "net" resorption in the tubule is more efficient, as it leads to lower urinal excretion of potassium.

Circadian variations in Na, K, and Cl excretion were found in humans (2,9,10,19), in rats (23), and in dogs (24). Circadian rhythms of sodium, potassium, and chloride excretion observed in this research are confirmed by the research conducted by Aizman and Velikanov (25) on humans, which found that the excretion rhythm period of Na, K, and Cl is respectively 23.80 h, 23.50 h, and 23.30 h long. Skrzypczak et al. (26) found the day-night differences in renal activity of sodium, potassium, and chloride balance in 2-week-old calves. F, TR, and UV change tendencies

of Na and Cl ions were similar, whereas the changes in renal potassium conservation were opposite. The authors observed higher Na and Cl resorption during the day and lower rates at night. Especially high TR_K at night was the reason for the decrease in urinal excretion of the ion.

Aslanin et al. (27) found that circadian rhythms of plasma filtration from main osmotically active electrolytes in humans changed significantly depending on the season. They suggested that when analyzing diurnal renal activity, this factor should be taken into consideration. In our research, we did not find any significant influence of the feeding time on the existence of urinary electrolytes' excretion circadian rhythms. Aslanin et al. (10) proved, however, that the amplitudes of the rhythms depend on the feeding times, which was also confirmed by the research done by Markovitz et al. (28).

An alternate occurrence of "light" and "dark" periods is undoubtedly an outer synchronizer of the diurnal renal activity, including electrolytes' excretion. If cyclic "light–dark" phases do not occur, feeding time can be a potential synchronizer of the circadian variations (10). Thus, it seems that the observed circadian variations of Na, K, and Cl excretion are related to the animals' activity. This is confirmed in the literature by Markovitz et al. (28).

Animal physical activity influences circadian rhythms of hormones' synthesis and release. The hormones directly regulate the absorption and secretion of the electrolytes' processes (5–7).

Bultasova et al. (9) in their research on humans found that UV_{Na} changes diurnally and it increases in the morning (after awakening), reaching its maximum between 1200 and 1400 hours and decreasing later on, which corresponds to the circadian variations of aldosterone levels. The observed Na and K excretion rhythms' acrophases coincided with the GFR rhythm's acrophase, whereas UV_{Cl} rhythm acrophase had occurred before. Similarity of UV_{Na} and GFR rhythms' acrophases was also found by Koopman et al. (2). The authors showed, however, that the peak of potassium excretion occurs earlier than the glomerular filtration peak and the filtered load level of the ion. Moreover, it is correlated with K level rhythm acrophase in plasma. Koopman et al. (19) also claimed that the level of K excreted with the urine depends mainly on the resorption and secretion processes in renal tubules and less on GFR level, which corresponds to the results of our research.

The above facts lead to the conclusion that the circadian renal rhythm of electrolytes' excretion is the consequence of GFR diurnal variations and filtered load level dependent on the process. Resorption-secretion preprocesses in renal tubules may modify the variations, but to a minimal extent.

The results lead to the conclusion that diurnal renal variations in goats exist mainly in the form of circadian rhythms with clear periods, acrophases, and diverse amplitudes. This, and the solidity of the electrolyte extracellular fluid, including plasma, confirms the "adapting" character of the rhythms. Due to the circadian glomerular filtration variations, resorption and secretion processes in nephrons, and, in consequence, through the changes of the final urine composition changes, the kidneys lead to the organism's adaptation to changes in the environment and also in circadian rhythm.

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