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INVESTIGATIONS ON THE CONTENTS OF THE CECUM OF HORSES FED ON SUMMER AND WINTER FODDER

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Since the beginning of the XIX-th century many investigations were carried out on the cecum of horses. Ellenberger in 1879 revewed the investigations of the past and compared them with the results of his own observations of the cecum contents of slaughter horses fed previously on adequately choosen fodder [4]. He did not attribute to the cecal glands important digestive activities, and maintained that enzymes proceeding from small intestine glands as well as the presence of protozoa have great significance in the decomposition of less easily digested foods. Till the publication of Ellenberger's works, only function of water and minerals absorbtion was attributed to the horse cecum, and it was considered as a receptacle of non digested food remains. Ellenberger and Hofmeister studied enzymatic processes in the cecum [5]. But Scheunert and Krzywanek in 1906 basing on their own [13] and others' [7, 9] investigations, stated that chemical changes in the cecum should be attributed first of all to the rich bacterial flora which decompose cellulose. Sym's research [14] proved an activity of amylolytic, lipolitic and proteolitic enzyms in the horse's cecum. These processes occur in an alkaline environment. Sym [15] and Nyrek [12] observed that the pH of cecum contents of slaughter horses ranged about 7,1 when anaerobic conditions were preserved.

Investigations carried out on horses with permanent cecal fistulas by Howell and Cups [8], showed changes of reaction of cecal contents from pH 6,3 to 7,4 depending on the time space after the last feeding, and as these authors suppose, on the degree of acidity of the ingesta in the stomach. Aleksander [1] was of a different opinion and he considered that the increase of concentration of hydrogen ions in the cecum depends on the amount of fatty acids, which are a result of the bacterial decomposition of cellulose. The concentration of volatile fatty acids in the cecum is kept on higher level when horses are fed on green fodder. Aleksander

maintains, that daily pH fluctuations depend also on the time after feeding. During starvation the amount of volatile fatty acids in the contents of the cecum decreases about eight times (after 48 hours from 400,9 to 64,3 mg%). Aleksander using the chromatography method for estimation the fatty acids in the large intestine of horses, found about 68% of acetic acid, 24% of propionic acid and 7% of butyric acid. Studying the composition of cellulose this author found that its decay in the cecum exceeded three times that in the dorsal colon and twice that in the ventral colon.

The aim of this paper is the observation of the cecal contents of horses fed on summer and winter fodder.

EXPERIMENTAL METHODS

Investigations were carried out on two horses with permanent cecal fistulae. During the period of the experiment horses were not used for work and were stabled in a separate place [1]. The technique for cannulating the large intestine was described in the work of Koźniewski [10]. Samples from the cecum for the analyses were withdrown three times during the day: at 7 a. m. — 17 hours after the last feeding, at 10 a. m. — 3 hours after the morning feeding and at 1 p. m. — 6 hours after the morning feeding. In the centrifuged (during 15 minutes at 3000 r/m) liquid cecal contents, the total nitrogen was estimated by the Kjeldahl method, the protein nitrogen by the Bernstein method, amino nitrogen by the Van Slyke method, volatile fatty acids by the method used by Gray and co-workers [6], the pH on an electric pH-meter, and free amino acids by the method of uni- and two-dimensional partition paper chromatography. For the two-dimensional partition two solvents have been used: phenol of pH 12 and then butanol with water and acetic acid in the proportion 4 : 5 : 1. Uni-dimensional chromatograms were developed four times in the same direction in butanol with water and acetic acid in the proportion 4 : 1 : 1, each time after drying of the solvent. For colour development of chromatograms, spraying with 0,2 percent alcohol solution of ninhydrin was applied. Spots were identified by means of standard solutions of individual amino acids.

The experiment comprised two periods:

I. September 1959 — horses were fed only on green alfalfa (summer feeding)

II. February 1960 — horses were fed on squashed oats and hay (winter feeding).

[1] For cannulating the cecum the operation hall of the Surgical Clinic was used, and the horses after the operation were kept in the Internal Clinic of the Veterinary Department instead of at the Experimental Station in Brwinów.

RESULTS

The figures given in the Tables are averages from six analyses.

Total nitrogen. The amount of total nitrogen in the cecal contents of horses was given in Table 1.

Table 1. Average total nitrogen in the cecal contents of horses (mg%)

	Period I — summer feeding			Period II — winter feeding		
Hours	7 a. m.	10 a. m.	1 p. m.	7 a. m.	10 a. m.	1 p. m.
mg%	20,4	21,1	23,5	41,5	24,7	26,0

It results from the figures quoted in Table I, that in the period I (alfalfa fodder), the lowest amount of total nitrogen in the cecal contents of horses was noted at 7 a. m. that is 17 hours afer the las feeding. In 3 and 6 hours after the morning feeding, the amount of total nitrogen gradually increased. In period II (winter feeding), just the opposite happened than in period I, the highest amount of total nitrogen was found at 7. a. m. At 10 a. m. its decrease and at 1 p. m. a small increase was noticed. Furthermore in period II at 7 a. m. the amount of total nitrogen was considerably higher than at any time in period I.

Fig. 1. represents daily fluctuations of the amount of total nitrogen in the cecal contents of horses.

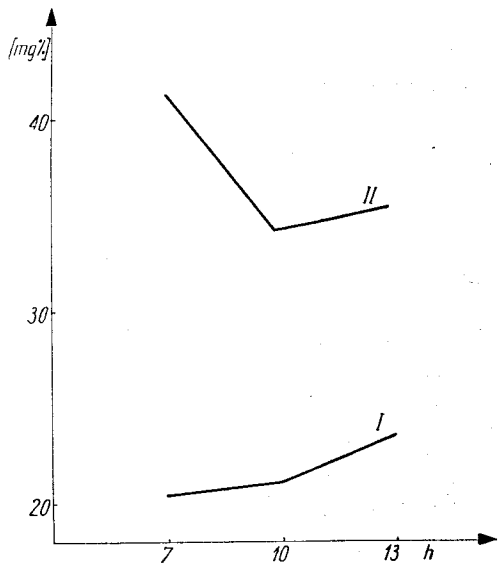


Fig. 1. Average daily fluctuations of the amount of total nitrogen in the cecal contents of horses fed on summer and winter fodder.

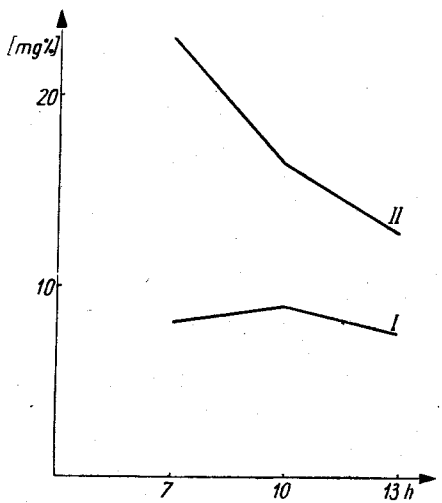


Fig. 2. Average daily fluctuations of the amount of protein nitrogen in the cecal contents of horses fed on summer and winter fodder.

Protein nitrogen. The amount of protein nitrogen in the cecal contents of horses was given in Table II.

Table 2. Average protein nitrogen in the cecal contents of horses (mg%)

	Period I — summer feeding			Period II — winter feeding		
Hours	7 a. m.	10 a. m.	1 p. m.	7 a. m.	10 a. m.	1 p. m.
mg%	8,1	9,0	7,6	23,0	16,4	12,9

Examinning data presented in Table II we may state in period I (feeding with alfalfa) a small increase of protein nitrogen at 10 a. m. and then its decrease at 1 p. m. In period II (winter feeding) a regular, gradual decrease of protein nitrogen at 10 a. m. and 1 p. m., when compared with the amount at 7 a. m., was observed. It was also stated, that the quantity of protein nitrogen in the contents of cecum was larger when horses were fed with winter fodder than with summer fodder.

Fig. 2 represents daily fluctuations in the amount of protein nitrogen in the cecal contents of horses.

Amino nitrogen. Table III contains the amount of amino nitrogen in the cecal contents of horses.

Table 3. Average amino nitrogen in the cecal contents of horses (mg%)

	Period I — summer feeding			Period II — winter feeding		
Hours	7 a. m.	10 a. m.	1 p. m.	7 a. m.	10 a. m.	1 p. m.
mg%	3,15	3,85	2,06	7,66	8,72	5,48

It results from the figures presented in Table III, that in period I as well as in period II, i. e. on summer and winter fodder, an increase of amino nitrogen in the cecal contents of horses three hours after feeding (at 10 a. m.), in comparison with the amount at 7 a. m., was noted. At 1 p. m. that is six hours after feeding, a decrease of amino nitrogen was observed. A larger amount of amino nitrogen in the cecal contents of horses was observed in the winter period than in the summer one.

Daily fluctuations of the amount of amino nitrogen in the cecal contents of horses are shown in Fig. 3.

Free amino acids. Using the method of uni- and two-dimensional partition paper chromatography eleven free amino acids were detected in the cecal contents of horses in both periods of the experiment, namely: glu-

tamic acid, alanine, valine, leucine, aspartic acid, threonine, glycine, serine, tyrosine, phenylalanine and derived from cystine. The intensity of individual amino acids spot colouring was marked by means of a 10 grade visual scale (Dent and Schilling [3]) and plotted in Table IV.

It results from Table IV that the most intensely coloured spots, in both periods of the experiment, were formed by glutamic acid, alanine and next valine. The spots of all individual amino acids were less intensely coloured when horses were fed with green alfalfa than with winter fodder. In the

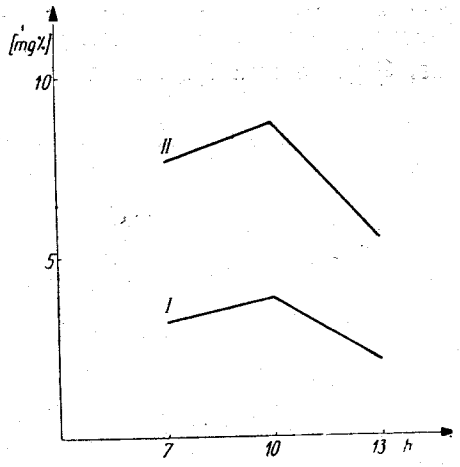
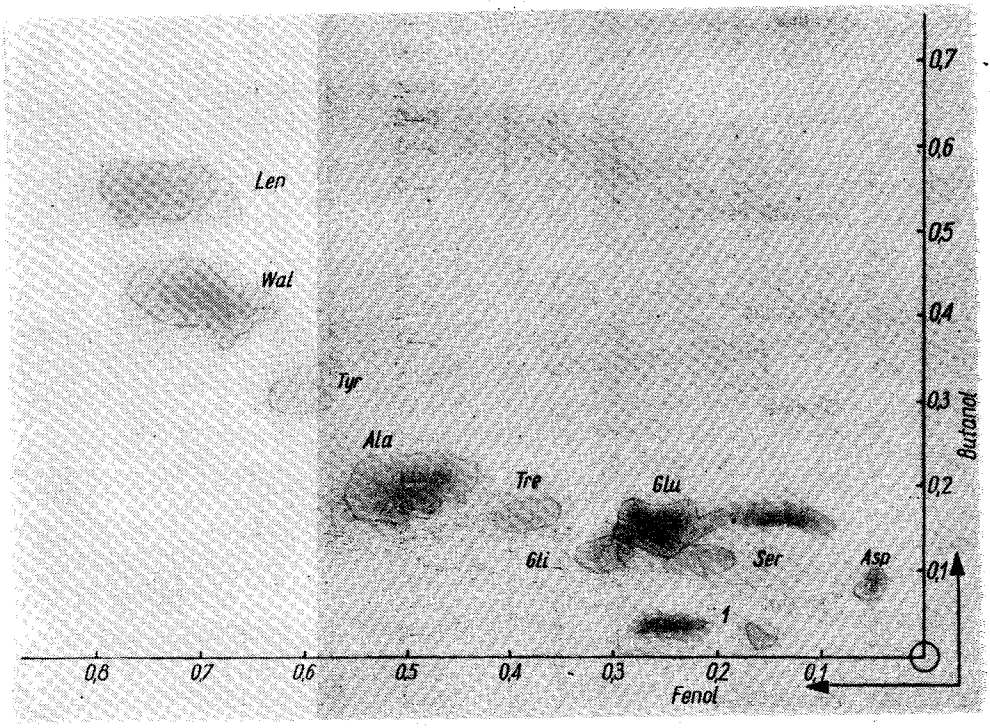


Fig. 3. Average daily fluctuations of the amount of amino nitrogen in the cecal contents of horses fed on summer and winter fodder.



Picture 1. Two-dimensional chromatogram of free amino acids of the cecal contents of the horse fed on winter fodder (Leu — leucine, Wal — valine, Tyr — tyrosine, Ala — alanine, Tre — threonine, Glu — glutamic acid, Gli — glycine, Ser — serine, Asp — aspartic acid, 1 — cystine derivates).

first period of the experiment (summer fodder) only three amino acids appeared on all chromatograms: glutamic acid, alanine and valine. Glycine and tyrosine were detected on 15 chromatograms, serine

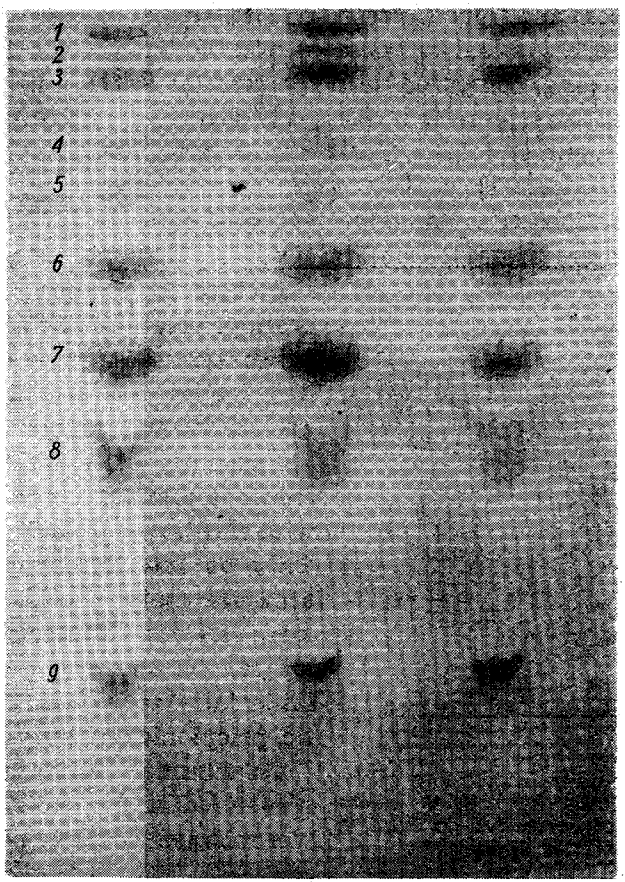
Table 4. Free amino acids of the cecal contents of the horse fed on summer and winter fodder

Amino acid	Hour	Fluctuations in the intensity of spot colouring	
		Summer period	Winter period
Glutamic acid	7	3—5	4—6
	10	4—5	4—6
	13	3—4	4—5
Alanine	7	3—5	4—5
	10	3—5	4—6
	13	3—4	4—5
Valine	7	2—3	3—4
	10	3—4	3—4
	13	2—3	3—4
Leucine	7	1—2	1—2
	10	0—2	2—3
	13	0—1	1—2
Aspartic acid	7	0—2	1—2
	10	0—2	1—2
	13	0—2	1—2
Threonine	7	0—2	1—2
	10	0—2	1—2
	13	0—2	1—2
Glycine	7	0—2	1—2
	10	0—1	1—2
	13	0—1	0—2
Serine	7	0—1	1—2
	10	0—2	1—2
	13	0—2	0—2
Tyrosine	7	1—2	1—2
	10	0—1	1—2
	13	0—2	1—2
Phenylalanine	7	0—1	0—1
	10	0—1	0—1
	13	0—1	0—1
Derived from cystine	7	0—1	0—1
	10	0—1	0—1
	13	0—1	0—1

and threonine on 14, leucine on 13, aspartic acid on 11, derived from cystine on 9, and phenylalanine on 7 chromatograms from 17 developed. In the period II (winter fodder) 7 amino acids were detected on all chromatograms: glutamic acid, alanine, valine, leucine, aspartic acid, threonine and tyrosine. Glycine and serine appeared on 17, phenylalanine on 12 and the derived from cystine on 10 chromatograms out of 18 developed.

Picture 1 represents a two-dimensional chromatogram of amino acids from the cecal contents of horses fed with winter fodder.

Picture 2 represents a uni-dimensional chromatogram of amino acids from the cecal contents of horses fed with winter fodder.



1 2 3

Picture 2. Uni-dimensional chromatogram of free amino acids of the cecal contents of the horse fed on winter fodder (1 — leucine, 2 — phenylalanine, 3 — valine, 4 — tyrosine, 5 — non determined spot, 6 — alanine, 7 — glutamic acid + threonine, 8 — glycine + serine + aspartic acid, 9 — cystine derivates).
Chromatogram 1—7^{00}, 2—10^{00}, 3—13^{00} h.

Volatile fatty acids. The amount of volatile fatty acids in the cecal contents of horses is given in Table V.

Table 5. Average volatile fatty acids in the cecal contents of horses (miliequivalents per 100 ml. of centrifuged liquid contents

	Period I — summer feeding			Period II — winter feeding		
Hours	7 a. m.	10 a. m.	1 p. m.	7 a. m.	10 a.m.	1 p. m.
mg%	5,72	7,52	9,13	6,14	7,05	7,55

It results from the figures presented in Table V that in the cecal contents of horses a gradual increase of volatile fatty acids three and six hours after feeding, compared with the amount at 7 a. m., was stated in both periods of the experiment. Also, a higher level of volatile fatty acids was noted on summer fodder. These results are in agreement with Aleksander investigations [1].

Daily fluctuations of the amount of volatile fatty acids in the cecal contents of horses are presented on Fig. 4.

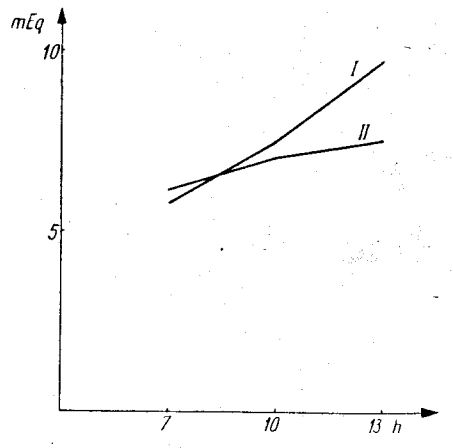


Fig. 4. Average daily fluctuations of the amount of volatile fatty acids in the cecal contents of horses fed on summer and winter fodder.

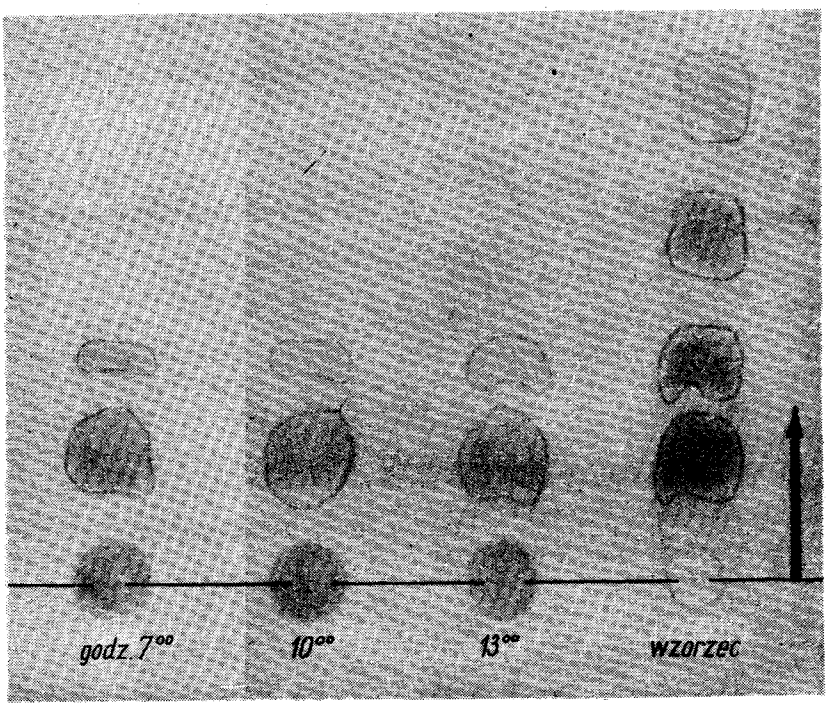
Picture 3 represents a chromatogram of volatile fatty acids of the cecal contents of a horse fed with summer fodder, and the picture 4 — of a horse fed with winter fodder.

Two fatty acids were detected on chromatograms when horses were fed with alfalfa: acetic and propionic acid. Only on few chromatograms appeared also butyric acid. When horses were fed with winter fodder on the majority of chromatograms three volatile fatty acids were detected: acetic, priopionic and butyric acid.

On summer fodder as well as on winter fodder the spots of acetic acid were always the most intensely coloured. Also in both periods of the experiment a gradual increase of the intensity of spot colouring (particularly of the acetic acid) was observed at 10 a. m. and 1 p. m. in comparision with 7 a. m. Similar results were obtained by Aleksander [1] who stated in the total amount of volatile fatty acids of the cecal contents of horses 68 percent of acetic acid.

pH values. In table 6 the pH values of the cecal contents of horses are compared.

It results from the data contained in Table VI, that on both fodder diets, a gradual decrease of pH values in the cecal contents three and six hours after feeding compared with the pH values at 7 a. m. (before feeding), was observed. This decrease is due to and probably results from the increase of amount of volatile fatty acids, which was noted in the present expe-

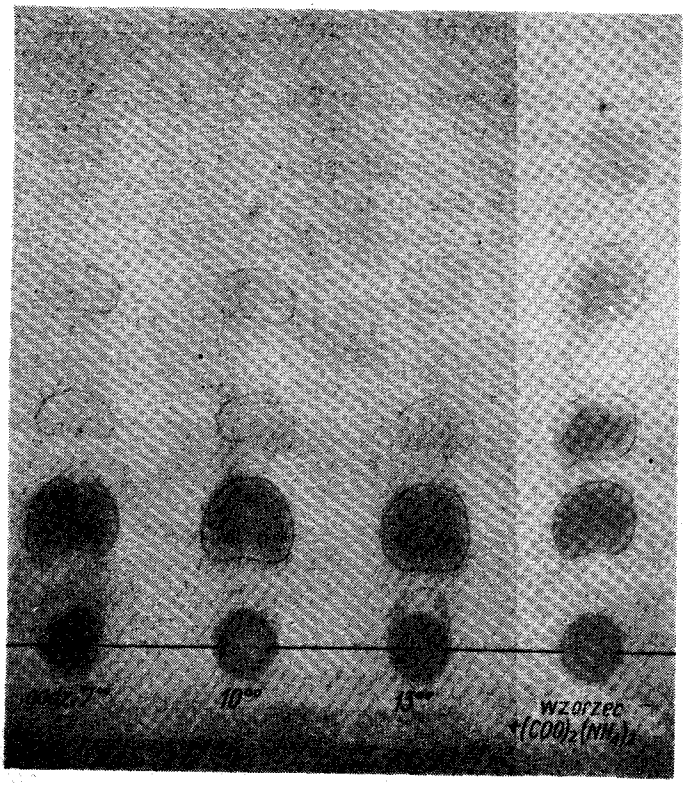


Picture 3. Chromatogram of volatile fatty acids of the cecal contents of the horse fed on summer fodder (successively from bottom to top: acetic, propionic, butyric and valeric acid). Wzorzec = standards, godz. = hours.

Table 6. Average pH values of the cecal contents of horses

	Period I — summer feeding			Period II — winter feeding		
Hours	7 a. m.	10 a. m.	1 p. m.	7 a. m.	10 a.m.	1 p. m.
pH	7,67	7,19	7,04	7,34	6,97	6,75

riment. These observations are in agreement with the investigations of Aleksander [1]. In his opinion the decrease of pH values in the cecal con-



Picture 4. Chromatogram of volatile fatty acids of the cecal contents of the horse fed on winter fodder (successively from bottom to top: acetic, propionic, butyric and valeric acid). Wzorzec = standards, godz. = hours.

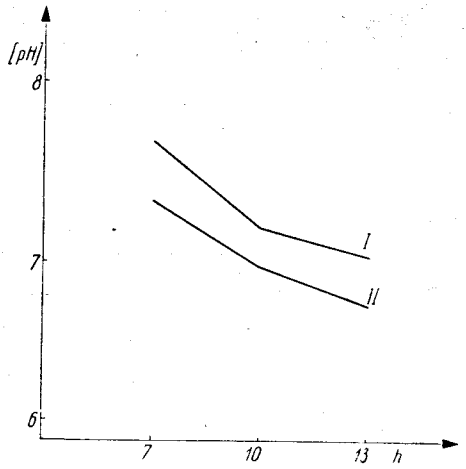


Fig. 5. Average pH values in the cecal contents of horses fed on summer and winter fodder.

tents of horses is caused by the accumulation of volatile fatty acids derived from the decomposition of cellulose.

Daily fluctuations of the pH values in the cecal contents of horses are presented in Fig. 5.

CONCLUSIONS

I. 1. In the cecal contents of horses fed on winter fodder a considerably higher amount of total-, protein- and amino nitrogen was found than in horses fed on summer fodder. This can be probably explained by the fact, that alfalfa is a fodder which is easier digested than oats and hay, and its nutritive ingredients are more rapidly absorbed.

2. Three hours after feeding an increase of total protein and amino nitrogen was stated in horses fed with summer fodder. On winter fodder in the same time an increase of amino nitrogen was only noted, and on the contrary a decrease of the amount of total and protein nitrogen.

II. 1. In both periods of the experiment, 11 free amino acids were detected in the cecal contents of horses.

2. Glutamic acid, alanine and valine formed the most intensely coloured spots.

3. Spots of all individual amino acids were less intensely coloured on summer fodder than on winter fodder.

4. On summer fodder only three amino acids were detected in the cecal contents (glutamic acid, alanine and valine), and on winter fodder seven (glutamic acid, alanine, valine, leucine, aspartic acid, threonine and tyrosine).

III. 1. In both periods of the experiment a gradual increase of the amount of volatile fatty acids three and six hours after feeding was stated.

2. Mostly two fatty acids were detected in the cecal contents of horses fed with summer fodder: acetic and propionic acid. In the cecal contents of horses fed with winter fodder — mostly three fatty acids: acetic, propionic and butyric acid.

3. In both periods of the experiment the most intensely coloured spot formed the acetic acid.

IV. 1. On winter as well as on summer fodder a decrease of pH values in the cecal contents of horses was observed three and six hours after feeding. This decrease was a result of an increase of volatile fatty acids.

2. The pH values were lower when horses were fed with winter fodder, than when they were fed with summer fodder.

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