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PHYSIOLOGICAL ASPECTS OF COMPLETE MIXED DIET DIGESTION IN COMPLEX STOMACH OF RUMINANTS ON THE EXAMPLE OF CATTLE (*Bos taurus taurus*)

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Abstract

Modern systems of normalized cattle feeding are based on the position that different feeding technologies do not affect the processes of digestion and absorption of nutrients of the same diet. In our studies, data were obtained for the first time, indicating changes in the parameters of the ration digestion process in the complex stomach of cattle, depending on the method of feeding: separate delivery of separate feeds and simultaneous delivery by the form of the Total Mixed Ration (TMR). To determine mechanisms of increasing feed dry matter intake in cattle when they were switched to Total Mixed Ration feeding, daily flux rates of chyme, its chemical composition and digestibility of main nutrient groups in the compound stomach of young cattle were studied. A Latin square design experiment was carried out using two diets differing in the forage fodder to concentrated fodder ratio and two methods of feeding (separate distribution of feed and in the form of TMR) by the method of periods with two Ukrainian red-motley bulls (*Bos taurus taurus*) of 300 kg live weight, with a sluice T-shaped duodenal canula. As a result of switching to the TMR feeding, both the nature of chyme influx to the duodenum and nutrient digestibility in the complex stomach were found to change. For 1 hour, the volume of chyme during TMR feeding increased by 417±71 ml/h as compared to separate high concentrate diet feeding, while compared with low concentrate feeding, it increased by 221±81 ml/h, or by 14.42 % and 8.36 %, respectively ($p < 0.001$). TMR increased Ca, P and total mineral residue influx to the duodenum (by 5.9 %, 10.1 and 8.5 %, respectively). At that, the increase in Ca with the first diet and in general ash with the second one was established at a level of trend, whereas for all the others the increase was significant ($p < 0.05$). This fact as well as the observed tendency to a change in the Ca and P concentrations in the duodenal chyme composition suggest an increase in the outflow of chyme from the rumen caused by both increased salivation and consumption of water by the animals. The feeding of animals with the TMR was shown to cause multidirectional changes in digestibility of all nutrients: digestibility of raw fat and crude protein decreased in both diets (by 39.6 % and 27.5 %, respectively), while digestibility of crude fiber increased (by 6.9 %); digestibility of nitrogen-free extractives did not change significantly in the first diet, whereas it tended to decrease in the second one so that the total digestibility of the dry and organic matter of the diet changed insignificantly. Raw fiber should be considered as an integral marker for assessing the TMR effect for rumen's digestion, since it is only digested in the rumen and is not synthesized by the microflora. Due to this, the increase in the chyme flow that we established, when we changed feeding method in the same type of diet, can reduce fiber digestibility due to accelerated outflow of rumen's content with small particles, whereas with the other type of diet, on the contrary, can increase digestion due to improvement of fermentation conditions.

Keywords: Ukrainian red-motley cattle, digestion, chyme volume, fiber, total mixed ration

Digestion of feed nutrients in ruminants can be divided into two processes. On the one hand, it is transformation of nutrients in the forestomachs where both the digestion of feed particles due to the enzymes produced by symbiotic microflora of the rumen and the synthesis of nutrients, constituting the microbial body itself, take place, on the other hand — digestion in the guts with own enzymes and the absorption of nutrients in the internal environment of the animal body. The second stage of digestion begins with the true stomach, which is the analog of non-ruminants' (monnogastric) stomach. The division into microbial digestion and own digestion is quite conditional: some products enter the internal environment of the macroorganism directly from the forestomachs, and part of the food is digested by the microflora of the small and large bowels [1, 2]. The main retention of feed masses occurs in the rumen since their promotion to the following bowel segments is possible only after grinding to a particle size of about 1 mm [3-5].

Many studies show [6-8] that changing to cattle feeding with Total Mixed Ration (TMR) increases the total intake of dry matter as compared to separate delivery of components of the same ration. The lack of selective feeding and reduced competitive relationships between animals are other positive effects [9-11]. We have previously revealed the effect of ration feeding technology on nutrient digestibility [12].

In this paper, we first obtained data on changes in the digestive process in young cattle depending on the mode of feeding the same ration. For example, the digestibility of raw fat and crude protein decreased in both high-concentrate and low-concentrate rations (by 39.6% and 27.5%, respectively), while the digestibility of crude fiber increased (by 6.9%); the digestibility of nitrogen-free extractives did not change significantly in the high-concentrate ration, whereas it tended to decrease in the low-concentrate ration.

The work objective was to study the dynamics of the daily flow of chyme, its chemical composition and digestibility of the main groups of nutrients in young cattle during switching to the Total Mixed Ration of feeding.

Techniques. The experiments (the physiological yard of Institute of Animal Science of the National Academy of Agrarian Sciences of Ukraine, 2015-2016) were carried out on castrated Ukrainian Red-and-White bulls (*Bos taurus taurus*), 300 kg bodyweight, with duodenal cannulas set at the beginning of the duodenum. The design of cannulas ensured the collection of chyme coming from the rennet into the duodenum, its assessment and return to the digestive system [13]. Animal were fed twice a day (at 8 a.m. and 5 p.m.) in equal portions. The chyme flow was measured for 9 hours after morning feeding. The obtained values of the chyme volume for 9 h were extrapolated to the daily interval, which made it possible to calculate the digestibility of nutrients in the complex stomach after the chemical analysis of selected samples of chyme and feed ration.

The effect of the Total Mixed Ration on the volume of duodenal chyme and the digestibility of nutrients in the complex stomach was studied according to the scheme of the Latin square with two rations differing in the ratio of voluminous and concentrate feeds in two periods on one group (2 animals). The volume of incoming chyme was measured in each animal in 3-fold repetition.

The available energy of rations was calculated by the formula:

$$AEC = 14.46 - 0.0007 \times CP + 0.0168 \times CF - 0.0192 \times CFb - 0.00028 \times NFE,$$

where CP, CF, CFb, NFE are the concentrations of crude protein, crude fat, crude fiber and nitrogen-free extractives in the ration, respectively, g/kg dry matter (DM), AEc is the available energy concentration, mJ/kg DM [14].

In the first period, hay and silage were given first, and dry concentrate feed was on top of the silage. Adaptation of young cattle digestion to the used ration occurred during 14 days. Then, the influx of chyme in the duodenum was measured during 14 days for a 9-hour interval after morning feeding (n = 6). Breaks for 1-2 days for the rest of animals were made between measurements. In the second period (28 days), pre-chopped hay, silage and mash were weighed and mixed (for each animal separately) with a drum mixer (manufactured in the laboratory of mechanization of livestock processes of the Institute of Animal Science of the NAASU). This technique ensured the delivery of all ration components in the form of TMR to each animal with the same accuracy as with separate feeding.

The chyme influx in the duodenum was measured as described above. Chemical analysis of the main nutrients of rations, chymus, and feces was carried as per standard techniques [15]. The animals were weighed before and after the experiment.

The Office Standard 2010 32-bit Russian software (license GGWA-A) (https://www.microsoft.com/ru-ru/downlo-ad/office.aspx) with dispersion analysis techniques was used for statistical calculations and drawing charts and diagrams. The arithmetic mean values (M), standard errors of means (±SEM), and mean square deviation (±σ) were calculated. The significance of differences was evaluated by paired Student's t-test (for allied series), which allowed elimination of the systemic error resulting from to individual peculiarities of animal digestion.

Results. Rations for test animals were made in such a way that their characteristics differed as much as possible (Table 1). This was made to assess the changes in the physiological parameters of digestion under the influence of TMR feeding vs. diametrically opposite conditions that occur in the rumen under low- and high-concentrate rations. Differences in energy and protein availability between rations were more than 20%.

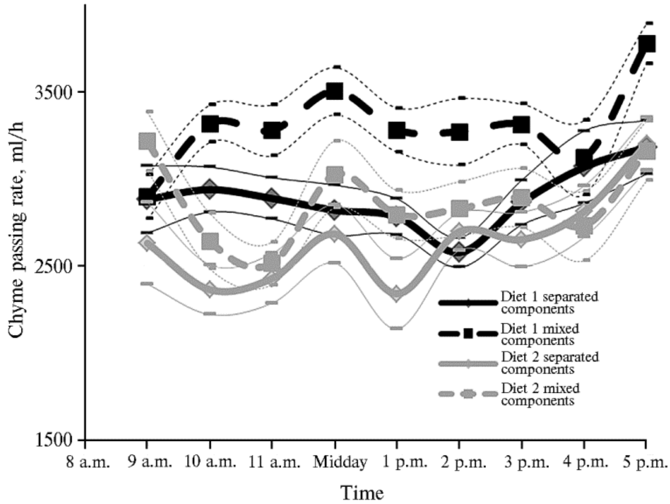
1. High- and low-concentrate rations in experiments on young Ukrainian Red-and-White cattle (*Bos taurus taurus*) (Institute of Animal Science of the National Academy of Agrarian Sciences of Ukraine, 2015-2016)

Ingredients, characterization	Ration		Comparison of rations (ration No. 2 to ration No. 1, %)
	high-concentrate (No. 1)	low-concentrate (No. 2)	
Corn silage, kg	11.00	16.80	
Alfalfa hay, kg	1.00	2.08	
Wheat groats, kg	0.30	–	
Corn groats, kg	0.30	–	
Sunflower cake, kg	3.30	–	
Barley groats, kg	–	0.72	
Mineral additive, kg	0.03	0.03	
Dry matter, g	7008	6355	90.68
Organic matter, g	6615	5996	90.65
Ash, g	393	359	91.28
Crude protein, g	1413	501	35.48
Crude fat, g	563	112	19.80
Crude fiber, g	1955	2170	111.01
Nitrogen-free extractives, g	2684	3213	119.74
Calcium, g	42.55	30.74	72.24
Phosphorus, g	25.62	21.18	82.67
Available energy, mJ	71.52	50.86	71.10
Available energy content, mJ/kg	10.21	8.00	78.41
Energy-protein ratio, g/mJ	19.76	9.86	49.89
Crude protein content, %	20.17	7.89	39.12
Basal metabolism provision, mJ/kg	1.03	0.63	61.56

N o t e. The basal metabolism provision is given per 1 kg of metabolic body weight (body weight to the power of 0.75, $W^{0.75}$). The dash indicates the absence of a component in the ration.

The average daily growth in body weight during the investigation was 1.12 and 0.26 kg with a swing of 0.95-1.29 and 0.21-0.32 kg, respectively, on rations No. 1 and No. 2, which is consistent with the literature data [16, 17].

The chyme influx from the complex stomach to the duodenum was uneven. At eating, the volume of chyme per time decreased compared to the period of animals' rest. Based on previous measurements, it was found that the volume of chyme for 9 hours after morning feeding was 37.47±0.97% with a coefficient of variation of 3.73% relative to the daily volume, which almost coincides with the time interval selected in the experiment: 9:24 × 100% = 37.5%. The chosen period facilitated the investigation greatly and, most importantly, reduced the stress impact on the operated animals.



Dynamics of chyme influx in the duodenum of young Ukrainian Red-and-White cattle (*Bos taurus taurus*) under separate feeding and feeding with Total Mixed Ration. For a description of the rations and conditions of the experiments, see the Techniques section (Institute of Animal Science of the National Academy of Agrarian Sciences of Ukraine, 2015-2016).

In switching to the TMR, the chyme influx in the duodenum and the digestibility of nutrients in the complex stomach changed. During the whole experiment, the rate of chyme influx from the rennet into the duodenum was higher when using the TMR (Fig.). At average, for 1 h, the chyme volume in feeding with the TMR vs. separate feeding increased by 417±71 ml (ration No. 1) and by 221±81 ml (ration No. 2), or by 14.42 and 8.36%, respectively ($p < 0.001$). With the TMR, not only significant changes in the volume of the chyme but also an increase in the flow rate of minerals from the complex stomach to the duodenum occurred in all cases.

Feeding the TMR increased the intake of calcium, phosphorus, and total mineral residue in the duodenum (Table 2). At the same time, an increase in the amount of Ca for ration No. 1 and total ash for ration No. 2 was found to be insignificant, at the trend level ($0.1 > p > 0.05$), for all other indicators, the increase was significant ($p < 0.05$). The concentration of minerals in the incoming chyme, except of Ca, decreased during the switching to TMR No. 2 while overall mineralization of the duodenal chymus markedly increased. Hence, it can be concluded that feed in the TMR form to a greater extent stimulates the flow of water into the complex stomach.

2. Mineral substances in the chyme in the initial section of the duodenum of young Ukrainian Red-and-White cattle (*Bos taurus taurus*) under separate and Total Mixed Ration of feeding (Institute of Animal Science of the National Academy of Agrarian Sciences of Ukraine, 2015-2016)

Ration	Separate feeding			Total mixed ration		
	Ca	P	ash	Ca	P	ash
Came to the duodenum, g/day						
No. 1	60.71	48.99	602.5	64.27*	53.94**	653.5**
No. 2	60.35	38.70	615.3	71.75***	42.16****	669.5
Minerals concentration in the chyme, mg%						
No. 1	87.68	70.51	868.0	81.75**	68.57	832.4****
No. 2	96.88	61.94	982.1	104.51***	61.03*	971.0

Note. For a description of the rations and conditions of the experiments, see the Techniques section.
* Differences for the Total Mixed Ration with indicators at separate feeding are statistically insignificant ($0.1 > p > 0.05$).
, *, **** Differences for the Total Mixed Ration with indicators at separate feeding are statistically significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

Variance analysis (Table 3) revealed a noticeable effect (more than 50% on the Chaddock scale) of the feeding mode on the intake of P (both rations) and Ca (ration No. 2) in the duodenum. At the same time, the studied factors (the composition of the ration and the form of feeding) influenced the total content of the mineral residue in the duodenum chyme.

3. Variance analysis of the influence of the feeding mode and the composition of rations on the intake of minerals in the duodenal chyme of young Ukrainian Red-and-White bulls (*Bos taurus taurus*) (Institute of Animal Science of the National Academy of Agrarian Sciences of Ukraine, 2015-2016)

Factor	Influence, %		
	Ca	P	ash
Ration composition			
Ration No. 1	10.5	57.8	12.3
Ration No. 2	59.2	52.8	13.7
Feeding mode			
Separate feeding	23.3	48.8	12.8
Total mixed ration	46.4	61.8	13.2

Note. For a description of the rations and conditions of the experiments, see the Techniques section.

According to the data on the salivary secretions in cows [18-20], Na prevails among cations, comprising 126 mEq/l (up to 85%), while K concentration is only 6 mEq/l (4%). The intake of Ca and Mg with saliva is less important for ionic equilibrium. Concentration of Ca ions is of 0.2 to 0.5 mEq/l. Among anions, bicarbonates (103-125 mEq/l) and phosphates (25-64 mEq/l) are of particular importance, determining the alkaline properties of saliva [18, 21]. High alkalinity, due to the content of bicarbonates and phosphates, is necessary to neutralize the acids formed in the pancreas as a result of fermentation. The composition of saliva is characterized by relative constancy [22-24], and changes in the intake of its components are associated with changes in the total amount of saliva. Apparently, it happens due to the fact that saliva has a micellar structure. The micelle consists of a nucleus, which is based on calcium phosphate. The nucleus is surrounded by hydrogen phosphate ions and an outer diffuse shell based on calcium ions [25].

Based on a significant increase in the intake of P in the duodenum for both rations, Ca for ration No. 2 and total ash for ration No. 1 (see Table 2), it can be assumed that the increase in the chyme outflow from the rumen found in our experiments is associated with increased salivation. At the same time, the data obtained on a certain decrease in the concentration of the studied minerals indicate an increase in the intake of water into the rumen in addition to its intake in the composition of saliva. Due to the lack of data on the dynamics of water consumption by animals, it is not possible to draw a conclusion about the origin of its additional amount. The increase in the consumption and flow of water directly through the rumen wall is highly likely. The very fact of dilution of the rumen fluid indicates rather an increase in water consumption since its flow from the intercellular fluid is likely to be accompanied by an increase in the number of electrolytes in the rumen content without the effect of dilution.

Some authors observed the effect of increased outflow rate associated with increased water consumption when using granular feed [26]. Given the fact that a significant amount of fluid is absorbed during the passage of the ruminal digesta through the omasum, and the flow of electrolytes from the gastric secretion into the rennet to some extent should smooth the effect of diluting the ruminal digesta with water drunk, then the increase in the flow of water into the rumen in the case of TMR feeding can be much higher than we have revealed. It is also known about the function of the omasum in regulating water-salt balance of the fluid coming from the rumen. Consequently, the fact of dilution of the ruminal fluid in the duodenal indicates a significant influence of the TMR on the water-salt balance in the rumen. The probable cause of the effect of dilution of Ca and P in the duodenal chyme we observed could be that the regulation of the body water balance is carried out by Na-dependent receptors [27, 28], and the maintenance of the water-salt balance in the digestive tract is mainly due to Na ions. An indirect confirmation can be the absence of any noticeable influence of both the ration and the feeding method on the amount of total ash in the duodenal contents (see Table 3).

As a result of switching from separate feeding to TMR, multidirectional changes in the digestibility of all nutrients in the complex stomach occurred but due to mutual compensation, the total digestibility of dry and organic matter of the ration changed slightly (Table 4).

4. Digestibility (%) of the main groups of nutrients in the complex stomach of young Ukrainian Red-and-White bulls (*Bos taurus taurus*) (*n* = 6, *M*±SEM, Institute of Animal Science of the National Academy of Agrarian Sciences of Ukraine, 2015-2016)

Ration	Indicator					
	DM	OM	CF	CP	CFb	NFE
	Separate feeding					
No. 1	50.09±1.19	56.23±1.90	18.40±18.38	44.62±3.77	70.50±2.35	61.07±2.60
No. 2	52.44±1.76	60.98±1.43	–64.04±27.87	–15.28±32.60	76.26±2.79	66.64±3.48
	Total mixed ration					
No. 1	48.75±3.16**	55.32±2.91	11.11±5.03*	32.34±7.06**	75.40±4.40**	62.07±3.75
No. 2	50.37±1.40*	60.06±1.13*	–44.98±8.18	–36.19±7.42**	78.67±3.03	64.19±2.66

Note. DM — dry matter, OM — organic matter, CP — crude protein, — crude fat, CFb — crude fiber, NFE — nitrogen-free extractives.
* Differences for the Total Mixed Ration with indicators at separate feeding are statistically insignificant (0.1 > p > 0.05).
** Differences for the Total Mixed Ration with indicators for separate feeding are statistically significant at p < 0.05.

As a result of changes in digestive processes under the TMR treatment, the digestibility of crude fat (CF) and crude protein (CP) decreased for both rations, and the digestibility of crude fiber (CF) increased. The digestibility of nitrogen-free extractives (NFE) for ration No. 1 did not change significantly, for ration No. 2 had a tendency to decrease. In our opinion, higher outflow rate of the liquid fraction from the rumen had a positive effect on the rumen microflora, especially cellulolytic. It is evidenced by significant differences in CP and CFb between separate feeding and TMR in the case of ration No. 1 and differences at the trend level ($0.1 > p > 0.05$) for ration No. 2. The active growth of the microbial populations of the rumen led to an increase in the amount of newly synthesized microbial CF and CP in the chyme coming from forestomachs. This had an impact on the decrease in the apparent digestibility of these groups of nutrients under TMR feeding for CF at the trend level, and for CP significantly ($p < 0.05$) (see Table 4). When using ration No. 2 with a low level of concentrates and a significant proportion of bulky feeds, the synthesis of microbial CF and CP exceeded the consumption of these nutrients with feeds. In this case, it is more correct to assess not the digestibility, but the availability of nutrients for digestion in the small bowel, i.e., the amount of nutrients received in the duodenum, in relation to those consumed with food. CF availability for ration No. 2 was 156% at separate feeding, 147% at TMR; CP availability was 120% and 138%, respectively.

The increased amount of CF coming from the complex stomach to the duodenum, compared to the content in the consumed feed, was associated with the transformation of some substances due to activity of the microbial population of the rumen. In this case, more fats, newly synthesized from carbohydrates and carbohydrate skeletons after deamination of amino acids, were produced compared to the content in the consumed feed. The increased content of nitrogenous substances in the chyme coming to the duodenum was also due to microbial synthesis, but the origin of additional nitrogen compared to the incoming feed is explained by its recycling inside the body.

As a result of deamination of amino acids in the process of basic metabolism, ammonia, toxic to the cell, is formed in the body tissues. For its neutralization, an energy-consuming mechanism of urea synthesis was developed based on three free amino groups [29]. It should be noted that the synthesis of urea occurs not only in the liver of animals, but also in the rumen wall directly [30-32]. The urea synthesized in this way enters the blood and forms a so-called pool of urea, from which it is partially directed to the rumen, and partially excreted in the urine. The main mechanism for guiding the urea in the rumen is its excretion with saliva. When it enters the rumen, urea is destroyed by ureases of the microflora to ammonia, which is used in microbial synthesis for the formation of amino acids and the subsequent synthesis of microbial and protozoal protein. The excretion of urea is most effective in the parotid gland (20-30 mg%), the blood concentration of this compound is 10-15 mg%, the concentration in the secret of the submandibular and sublingual glands is 10-15 and 8-10 mg%, respectively [33]. Depending on the intensity and uniformity of urea formation during the day, the ratio of its recycling and excretion may vary significantly. A significant role in this is played by the energy of the ration available for the microflora, especially digestible carbohydrates [34].

Based on our findings, both the feed factor and the intensity of urea entering the rumen as part of saliva affect the synthesis of microbial CP. Thus, in the case of ration No. 1 after the transition to TMR, the digestibility of CP in the rumen significantly increased, which indicates a decrease in the involvement of urea in nitrogen recycling and its more active excretion from the body. In other words, the amount of energy was not enough for microbial crude protein synthesis from nitrogen available in the rumen. When using ration No. 2, the potential supply of available energy to the microflora was higher, as a consequence, endogenous nitrogen was also involved in the microbial synthesis. Increased salivation caused by the TMR and the associated increased urea intake into the rumen contributed to the additional synthesis of microbial CP, which resulted in an increase in CP intake into the duodenum.

It has already been noted before that the increased outflow of fluid from the complex stomach during switching to the TMR positively affected the microbial population of the rumen. This was observed both on the high-concentrate ration, when the increased acidity due to the synthesis of a significant amount of propionic acid was noted, and on the low-concentrate ration with a predominance of the acetic-acid type of fermentation, typical for the digestion of fibrous foods rich in fiber. However, this mechanism had some differences depending on the composition of the ration. Increased fluid outflow from the rumen regulated microbial synthesis by reducing the concentration of microflora-born products in the first case, and by increasing the amount of the substrates used in the second case.

It should be taken into account that with the increased fluid outflow from the rumen, the removal of finely divided insoluble fraction of feed particles is accelerated. It can be assumed that increasing this fractional outflow would reduce the time of microbial fermentation of feed particles, which would inevitably lead to a decrease in the apparent digestibility of nutrients. In our experiments, we revealed the increase in the digestibility of NFE with a decrease in the digestibility of CF for ration No. 1 and a reduced digestibility of NFE with an increase in digestibility of CF for ration No. 2. Apparently, the increase in the intensity of microbial fermentation in one case was quite high and prevailed over the decrease in digestibility due to an increased outflow rate, and in the other, on the contrary, the microbial synthesis did not compensate a decrease in digestibility because of an increase in the fractional outflow rate, which affected the increase in the overall apparent digestibility.

Another mechanism of the influence of increased salivation on microbial fermentation in the rumen which is difficult to take into account is the fact that in ruminants, saliva has a relatively low surface tension (almost 1.5 times lower than in water). This property of saliva prevents the formation of a foamy mass in the rumen and mesh. The low surface tension of saliva has a detrimental effect on particular species of bacteria; therefore, it can participate in the regulation of the species composition of microorganisms inhabiting the forestomachs. This fact, in turn, affects the intensity of cleavage of certain components of plant food cells and the accumulation of newly synthesized microbial fat and protein in the rumen fluid.

The adequate interpretation of the obtained data is impossible without taking into account the fact that nutrients in the rumen are not only destroyed, but also, being synthesized again, accumulated in the composition of microbial biomass. To assess the interaction of the accelerated fractional outflow of small particles of feed and improved fermentation conditions due to the accelerated removal of microbial fermentation products, crude fiber should be considered as a marker since it is only digested in the rumen and not synthesized by microflora. In this regard, the increase in the chyme flow, revealed when changing the mode of feeding the same type of ration, may decrease the digestibility of fiber due to the accelerated outflow of small particles of the rumen content, whereas with another type of ration, on the contrary, its digestion may increase due to improved fermentation conditions (pH shift to the alkaline side).

Thus, in bulls of the Ukrainian Red-and-White breed, as a result of the switching to the Total Mixed Ration (TMR), the chyme influx into the duodenum and the digestibility of nutrients in the complex stomach changed. The chyme volume for TMR with high energy content increased by 417±71 ml/h, with low energy content by 221±81 ml/h. The TMR treatment increased the intake of calcium, phosphorus, and total mineral residue in the duodenum. The established increase in the intake of mineral components in the duodenal chyme and the change in their concentration indicate that switching to the TMR increases the outflow of fluid from the complex stomach both due to increased salivation and due to other mechanisms of increasing water intake into the complex stomach. The digestibility of all nutrients in the complex stomach when feeding the TMR varies in different directions. For crude fat and crude protein the digestibility decreases at both rations and for crude fiber it increases; no significant change occurs for nitrogen-free extractives at the high concentrate ration and a trend to the decrease appears at the low concentrate ration. As a result, total digestibility of dry matter and organic matter of the ration show a slight change. It is possible to use the index of digestibility of crude fiber as an integral marker for assessing the impact of the TMR on rumen digestion.

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