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Blessed memory of our Teacher
Vasily V. Tsyupko is devoted

FOOD PARTICLE SIZE AS AN INDICATOR OF ITS STRUCTURAL COMPOSITION AND A KEY ASPECT OF THE DEVELOPMENT OF THE NUTRITION THEORY PARADIGM

N.V. VASILEVSKY, T.A. YELETSKAYA

Institute of Animal Science of National academy of agrarian sciences of Ukraine, 3, 7-i Gvardeiskoi Armii st., Kharkov, 61120 Ukraine, e-mail vasilevskii.n@mail.ru (✉ corresponding author), eletskatat@yandex.ru
ORCID:
Vasilevskiy N.V. orcid.org/0000-0002-7437-2910 Yeletskaya T.A. orcid.org/0000-0001-8980-6972
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Abstract

The using of modern feed-preparation machines and new technologies for the preparation and feeding of rations in animal husbandry has exacerbated the problem of the ratio in food of relatively large particles that capable to support the necessary motility of the digestive tract and relatively small ones, which provide high consumption of dry matter and its digestibility. In the presented work, we were the first to obtain data about the relationship between the particle size of the diet, the contents of the gastrointestinal tract and the feces of cattle. Based on the determination of the average particle size and dry matter content, a method for assessing the structure of feed has been proposed. The concepts of the informational component of food and the definition of rationed feeding have been introduced into the paradigm of nutrition theory. The aim of the work was to study the impact of the particle size of the diet on the particle size of the contents of the gastrointestinal tract and feces of cattle; to assess the relationship of these indicators with the digestibility of the main groups of nutrients; to develop a methodological approach to rationing the diet structure; to supplement the theory of rationed feeding with the necessary concepts. The studies were carried out at the Institute of Animal Science of National academy of agrarian sciences of Ukraine in 2017-2019 on two heifers (*Bos taurus taurus*) of the Ukrainian red-and-white breed with a live weight of 350 kg fitted with a duodenal lockable cannula of the beginning of the duodenum and a large-diameter rumen cannula. The animals were fed twice a day (at 8 a.m. and 5 p.m.) in equal shares. Chyme flux was measured during 9 h after morning feeding. The intake of chyme into the duodenum was measured within 14 days ($n = 6$). The measurements were interrupted for 1-2 days to give the animals rest. Samples of the contents of the rumen were collected through a cannula with a 100 mm diameter from the middle part of the rumen mat in triplicate with a 3 h interval between morning and evening feeding. The experiments were designed according to the balance test methodology with simultaneous assessment of the duodenal chyme flux and sampling of the rumen contents, duodenal chyme and feces. The main diet consisted of hay, silage, concentrates and provided the maintenance of the basal metabolism. The other rations were obtained by replacing one or two components of the main ration with additional amounts of the remaining components in an amount that was equal to the dry matter value of the interchangeable feed. The average particle size of the feed particles was determined by the arithmetic weighted average method by manual parsing and dry sieving, and the selected biological samples by the wet sieving method. The metabolic energy of the rations was calculated. It was found that the average particle size of rumen, chyme and feces decreased with an increase in the average particle size of the diet. The average particle size of the rumen (8.68±0.49 mm, $n = 31$) was an order of magnitude higher than the average particle size of the duodenal chyme and feces, that indicates the most intense crushing of feed in the proventriculus. The average particle size of feces (1.09±0.06 mm, $n = 19$, $0.1 > p > 0.05$) exceeded the average particle size of the duodenal chyme (0.99±0.05 mm, $n = 31$), that is indicative of the predominant digestion of the small fraction in the intestine and the formation of feces from the large fraction of the chyme. The value of feed structure in our studies was 0.12-0.92 kg · m/day vs. 0.033-0.062 kg · m/day for the contents of the rumen, 0.0031-0.0057 kg · m/day for duodenal chyme, and 0.0019-0.0028 kg · m/day for feces. A relationship between the structure of the diet, the

availability of crude protein for digestion in the intestine and the size of feces particles was revealed, that theoretically suggests the possibility of assessing the state of digestive processes which based on the granulometric parameters of feces. The obtained data allow us to discuss the factors, affecting feed (and food) digestion and assimilation, which cannot be evaluated in a classical balance experiment by the difference in feed consumption and excretion. It has been proposed to collectively denote these factors as an information component which should be incorporated to express the concept of complex feed (food) structure. The information component is represented in part by various biologically active substances that are ingredients of the feed itself and/or products of the gastrointestinal microorganisms. Another part comprises physical factors, such as the physical parameters of food (temperature, humidity, particle size and stiffness), and non-feed factors (frequency and regularity of feeding, phased feeding, sound, light and other wave effects). We suggest the definition for rationed nutrition as a process of matter and information exchange between the external environment and the body, which ensures welfare and productive life.

Keywords: average particle size of food, structure of food, informational component of food, normalized feeding, theory of nutrition

A fully mixed ration technology is now widely used in the practice of feeding cattle [1, 2]. Possessing a number of indisputable advantages, this technology includes several important aspects the ignoring of which can negatively affect the health and productivity performance of animals. First of all, this concerns the uniformity of mixing and the degree of crushing of high fiber coarse feed [3]. Poorly chopped parts of feed do not allow the necessary mixing uniformity to be achieved which is required to exclude the selective eating of individual components of the mixture by animals [4]. Excessive grinding leads to weaker response of the receptors to feed and, as a result, to a decrease in the rate of digestion and movement of masses along the digestive tract, up to digestive disorders [5-7]. The concept of food structure is used to assess the ability of the daily ration to maintain the necessary stimulation of the motility of the digestive tract. The effect of structure on the intensity of digestion can be characterized by the degree of feed crushing expressed through the average particle size of the feed; frequency and duration of rumination and chewing; the density of the scar mat; the degree of digestion of dry matter; the content of neutral-detergent, acid-detergent or crude fiber [8)]. Some researchers use dimensionless indicators, e.g. percentages of or proportions to the reference feed [9].

This work for the first time shows data reflecting the relationship between the sizes of particles in the diet, in the gastrointestinal tract contents and in feces of cattle. A method for assessing the structure of feed is proposed which is based on the average particle size and dry matter content determination. These data allow us formulate a concept of the informational component of food and the definition of rationed feeding to be introduced into the paradigm of nutrition theory.

The objectives of the research were to reveal the effect of the particle size of the diet on the particle size in the contents of the gastrointestinal tract and feces in cattle, to assess the relationship of these indicators with the digestibility of the main groups of nutrients, to develop a methodological approach to the regulation of the structure of the diet, and to incorporate new definitions and concepts into the theory of rationed feeding.

Materials and methods. Two Ukrainian red-and-white heifers (*Bos taurus taurus*), 350 kg live weight, with a duodenal lockable cannula in the superior part of duodenum and a large-diameter ruminal cannula were involved in feeding trials (the Institute of Animal Science of the Ukrainian Academy of Agrarian Sciences, 2017-2019). The design of the cannulas ensured collection of chyme coming from the abomasum into the duodenum, its quantitation and return to the digestive system [10]. The animals were fed twice a day (at 8.00 and 17.00) in equal portions. Chyme flux was measured for 9 hours after morning feeding. The obtained values of the chyme volume for 9 hours were extrapolated to the daily interval,

which made it possible to calculate the digestibility of nutrients in the complex stomach after chemical analysis of the selected chyme samples and feed.

The adaptation of the digestion to the tested diet took 14 days. Then, within 14 days, the intake of chyme into the duodenum was measured (n = 6). Between measurements, the animals were allowed for 1-2-day rest. Rumen contents were sampled through a 100 mm cannula from the middle part of the rumen mat in 3 replicates with a 3-hour interval between morning and evening feeding. The experiments were carried out according to the methodology of balance experiments with a simultaneous assessment of the duodenal chyme flow and collection of rumen contents, duodenal chyme and feces.

Composition of feed, the gastrointestinal tract contents and feces was assessed using common analytical methods [11]. The main diet consisted of hay, silage, concentrates and provided the maintenance of the basal metabolism. The rest of the rations were obtained by replacing one or two components of the main ration with an additional amount of the remaining components equal to the dry matter content of the interchangeable feeds. The designed scheme met the requirements of data statistical processing by the dispersion method to quantify the influence of individual dietary ingredients on the structure of chyme and feces, and on the digestibility of the main groups of nutrients.

For feed, the average size of particles was determined by the arithmetic weighted average method after manual particle separation and dry sieving, for the collected biological samples the wet sieving method was used [12].

Dietary metabolizable energy (ME) we calculated as

$$ME = 14.46 - 0.0007 \times CP + 0.0168 \times CF - 0.0192 \times CF^{*} - 0{,}00028 \times NFE,$$

where CP, CF, CF*, and NFE are crude proteins, crude fat, crude fiver and nitrogen-free extract (NFE), g/kg dietary dry matter (DM). The availability (b) of nutrients in the small intestine was calculated as

$$b = C/A \times 100\ \%,$$

where C is the amount of nutrient entered the small intestine per day, A is the amount of nutrient consumed with food per day [13].

Statistical calculations, construction of graphs and diagrams were performed using the licensed software package Office Standard 2010 32-bit Russian (license GGWA-A) (https://www.microsoft.com/ru-ru/download/office.aspx) by analysis of variance methods. The arithmetic mean values (M) and standard errors of the mean (±SEM) were calculated. The significance of differences was assessed using the paired Student's t-test and the conjugate series approach (direct difference).

Results. Tables 1 and 2 present the characterization of the six calculated diets that were fed to the heifers.

1. Composition (kg natural feed per day) **of the diets fed to Ukrainian red-and-white heifers (*Bos taurus taurus*)** (Institute of Animal Science of UAAS, Kharkov, 2017-2019)

Feed	Diet No.					
	1	2	3	4	5	6
Silage	16.00	20.00	24.40	19.74	–	–
Hay	2.00	3.00	–	–	8.39	4.14
Concentrates	2.00	–	–	2.96	–	4.04

N o t e. Dashes mean the absence of the ingredient in the diet.

The average particle size of the rumen content, duodenal chyme, and feces decreased as the average particle size of the diet increased (Fig. 1). An inverse relationship was found between the size of feed particles and the particle of the digestive tract content. The slope of the linear regression turned out to be close for chyme and feces (–0.0044 and –0.0048 mm/mm, respectively), for the rumen contents of the degree of the food particles size influence was an order

of magnitude higher (–0.0479). The average particle size of the feces was slightly higher than that of the duodenal chyme, 1.09±0.06 and 0.99±0.05 mm, respectively. Statistical processing of the entire array of particles showed a difference at the level of tendency in their sizes in chyme and feces ($0.1 > p > 0.05$). The conjugate series approach to the same data analysis revealed a significant difference ($p < 0.05$). Most likely, in the small and large intestines, digestion preferentially occurs, the relatively small fraction of chyme particles disappears, and the feces in the rectum are formed from the remaining larger fraction.

2. Characterization of the diets fed to Ukrainian red-and-white heifers (*Bos taurus taurus*) (Institute of Animal Science of UAAS, Kharkov, 2017-2019)

Diet No.	Dry matter, g	Crude fiber, g	Crude protein		Metabolizable energy		Basal metabolic energy supply, MJ/kg
			amount, g	concentration, %	total MJ	concentration, MJ/kg	
1	7685	1690	593	7.71	77.76	10.12	0.80
2	7894	2137	523	6.62	85.17	10.79	0.87
3	6666	1698	444	6.66	69.80	10.47	0.71
4	7899	1492	649	8.21	82.62	10.46	0.85
5	6797	2083	444	6.53	67.61	9.95	0.69
6	6775	1190	614	9.06	66.22	9.77	0.68

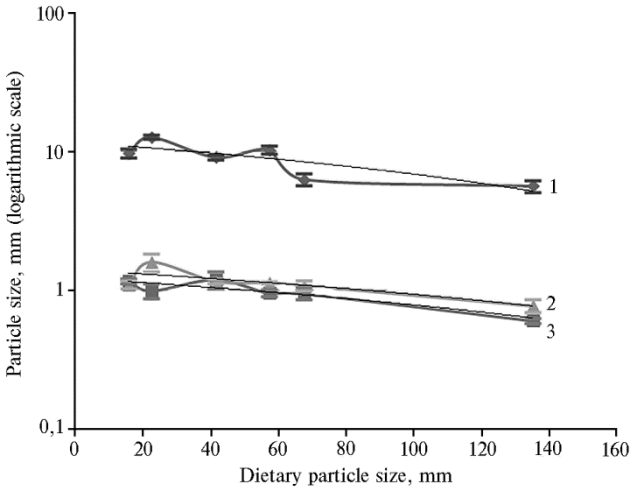

Fig. 1. Size of particle in the rumen content (1)**, chyme** (2) **and feces** (3) **of Ukrainian red-and-white heifers (*Bos taurus taurus*) depending on the dietary particle size** (*M*±SEM, Institute of Animal Science of UAAS, Kharkov, 2017-2019). The linear regressions: for rumen $y = –0.0479x + 11.624$, $R^2 = 0.6311$; for chyme $y = –0.0044x + 1.2185$, $R^2 = 0{,}806$; for feces $y = –0{,}0047x + 1{,}3979$, $R^2 = 0{,}5951$. For description of diets and design of the trial, see *Materials and methods.*

3. Particle size and structural orderliness of feed, gastrointestinal tract content and feces of Ukrainian red-and-white heifers (*Bos taurus taurus*) (*M*±SEM, Institute of Animal Science of UAAS, Kharkov, 2017-2019)

Sample	Diet No.					
	1	2	3	4	5	6
	Particle size, mm					
Feed	41.48	57.13	22.43	15.65	135.14	67.43
Rumen	9.05±0.32	10.26±0.67	12.61±0.36	9.69±0.75	5.60±0.55	6.28±0.62
Chyme	1.19±0.16	0.95±0.05	0.99±0.12	1.16±0.04	0.60±0.03	0.94±0.10
Feces	1.15±0.04	1.13±0.02	1.58±0.22	1.10±0.07	0.77±0.08	1.08±0.09
	Structural orderliness, kg·m/day					
Feed	0.319	0.451	0.150	0.120	0.919	0.457
Rumen	0.051	0.060	0.062	0.061	0.033	0.039
Chyme	0.0043	0.0036	0.0032	0.0057	0.0031	0.0052
Feces	0.0031	0.0025	0.0033	0.0030	0.0019	0.0029

Despite the fact that the average size of feed particles has a significant

effect on the activation of receptors in the digestive tract, it cannot serve as an adequate measure of feed structural orderliness, since the duration and degree of exposure also depend on the number of particles. In this regard, to assess the structural orderliness, we used an indicator calculated as the product of the average particle size (m) by the total amount of dry matter (kg) in the daily diet, the contents of the gastrointestinal tract, or feces (Table 3).

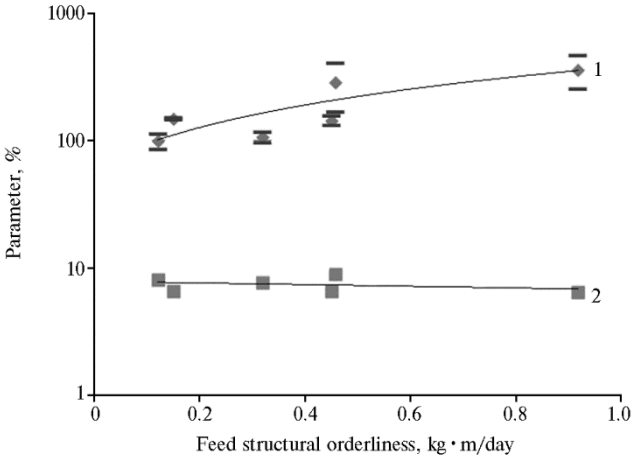

Fig. 2. Crude protein digestive availability in intestines (1) **and concentration** (2) **depending on the structural orderliness of feed fed to Ukrainian red-and-white heifers (*Bos taurus taurus*)** (*M*±SEM, Institute of Animal Science of UAAS, Kharkov, 2017-2019). The linear regressions for crude protein digestive availability y = 314.53x + 63.298, R^2 = 0.74, for crude protein concentration y = –1.101x + 7.9101, R^2 = 0,0946. For description of diets and design of the trial, see *Materials and methods.*

As structural orderliness increases in feed, it decreases in the rumen content and feces, with the degree of determination R^2 = 0.61 and R^2 = 0.86, respectively. Regression analysis of indicators of the feed structural orderliness, on the one hand, and feed digestibility in the rumen, as well as visible digestibility throughout the gastrointestinal tract, on the other, showed the lack of association for all major groups of nutrients, with the exception of crude protein (negative association, R^2 = 0.73 and R^2 = 0.74). Moreover, this did not become a consequence of some fluctuations in the content of crude protein in the diet (see Table 2), since changes in the structural orderliness of the diets and the amount of crude protein in feed did not interrelate (R^2 = 0.20). The intake of crude protein in the duodenum was higher than its amount taken with feed due to intensive microbial synthesis from endogenous nitrogen sources. A higher structural orderliness with a high degree of determination (R^2 = 0.74) led to an increase in the availability of crude protein for digestion in the intestine calculated as intake into the duodenum as a percentage of that consumed with the diet) (Fig. 2). The increase in the intake of crude protein in the intestine led to a slight decrease in its apparent digestibility throughout the gastrointestinal tract. Consequently, in the studied range of averaged feed particle sizes, a higher structural orderliness did not cause significant changes in the digestibility of the main groups of nutrients, with some improvement in the conditions for the synthesis of microbial crude protein in the rumen.

The availability of crude protein for intestinal digestion is an indicator of the intensity of microbial processes in the rumen. Its increase can also indirectly indicate the acceleration of fractional outflow from the rumen. That is, the structure of the diet, on the one hand, has a decisive effect on the structure and size of feces particles, on the other, on the availability of crude protein for

digestion in the intestine. Given this, we examined the relationship between the particle size of feces and the availability of crude protein for digestion in the intestine. The obtained value of the coefficient of determination ($R^2 = 0.37$) indicated a moderate degree of interdependence on the Cheddock scale between these indicators.

The obtained results do not allow us to identify the optimal range of the structural orderliness of the diet from the viewpoint of digestion conditions, since the energy consumption per unit of metabolic mass in the operated animals cannot reach the values typical for young fattening cattle, and even more so for lactating cows. The proposed methodological approach for assessing the structure of the diet makes it possible to correlate the indicators characterizing the digestive processes and the structural properties of feed. Research on highly productive animals is necessary to develop standardization of feed structure and rations for cattle. The main difficulty here is that the size and shape of particles cannot be considered within the framework of the theory of balanced nutrition as a food component, which, as a result of digestion and absorption, is assimilated by the body and consumed in the process of vital activity. Nevertheless, the effect of this factor on the digestion process does not raise doubts, and some modern rationing systems involve structural characteristics of feed and cattle diets [9, 14].

Studying the digestibility of nutrients from feed mixtures that are identical in chemical composition, but differ in the degree of grinding of roughage [15], we found a significant effect of the structural factor (particle size and their ability to resist mechanical grinding during chewing) on digestion in ruminants. However, the structural parameters of feed cannot be assessed in the classical balance experiment by the difference in consumption and excretion with feces and urine. The "apparently disappeared" structure is not absorbed into the internal environment of the body along with digested components determining structural charateristics, nevertheless, the "undigested structure" excreted in the feces has an effect on the digestive processes. Therefore, the concept of structural orderliness does not correspond to the main position of the theory of balanced nutrition, according to which the nutritional value of food is determined only by its digestible components.

Pokrovsky [16, 17] notes that nutrition is a complex process, the main goal of which is to ensure the growth and development of the child's body, maximum performance and well-being in adulthood, longevity and health in the elderly and in senile period. Nutrition is a source of aesthetic pleasure and an important healing factor [16, 17]. According to the author, it should not only compensate for the body's expenses to maintain vital functions (and in farm animals also productivity), but also provide an emotional and therapeutic component.

Ugolev et al. [18, 19] showed the participation of the gastrointestinal tract in the formation of the individual hormonal profile of the organism in a proportion reaching up to 50% of the total amount of hormones. This means that food, through the formation of a hormonal pattern, is involved in the regulation of gene expression, which means that it affects how a specific phenotypic image of an organism will be formed. In other words, in matters of nutrition, one should not be limited to only considering the role of nutrients in providing endogenous metabolic processes of the body with exogenous substrates; it is also necessary to recognize the regulatory function of food expressed at the neurohumoral level.

Within the framework of the existing paradigm of nutrition theory, it is impossible to explain the effect of phase feeding, which is sometimes used when fattening young cattle. The essence of the method is to periodically change the rate of feed delivery by 10-20%, alternately up and down from

100% of the need. This technique significantly increases live weight gain in comparison with a constant 100% ration delivery [20, 21].

Another example of the contradiction between the feeding practice and the theory of balanced (adequate) nutrition is the exchange of calcium in the body. It is known that the assimilation of this element is critically dependent on the availability of vitamin D [22, 23]. The synthesis of vitamin D, in turn, occurs when the body is exposed to ultraviolet radiation [24, 25]. That is, sunlight or other ultra-violet source should be counted as a "nutritive" element.

The current period in the development of nutrition science is characterized by a significant accumulation of facts that do not fit into the framework of the generally accepted concept, i.e. the classical theory of balanced nutrition. In our opinion, this information has reached a critical mass and prerequisites have been created for the formation of different views on the theory of nutrition and, accordingly, approaches to normalized feeding of animals. A.M. Ugolev [26] gives the most detailed criticism of the theory of balanced nutrition. Nevertheless, until now, there is no universally recognized alternative to the theory of balanced nutrition and, with the exception of some works [27]; most experts in animal nutrition rely on the paradigm of digestion and nutrient assimilation in the framework of the balanced nutrition theory. This is expressed, first of all, in the fact that in all modern systems of rationed feeding of farm animals the rationing is considered as compensation of the body expenses for maintaining vital activity and ensuring productivity.

The flow of substances vital for the body and coming from digested food components, in addition to traditional plastic and energy components, contains substances with zero and even negative energy value, the metabolization of which is associated with additional energy costs. Moreover, food has the ability to influence the body not only through nutrients, but also through other factors of a chemical and physical nature. Such an effect leads not only to changes in the digestion and assimilation of the components of the food itself, but also affects other vital functions of the body, up to a change in the hormonal profile and regulation of genome replication [18, 19, 26].

We guess that the described problems can be easily addressed if we recognize the existence of information flows directed both into the internal environment of the organism and from it. In this case, a special role in digestion is played by the exchange of signals with the enteric environment, which is partially separated from the "truly external". In our opinion, the paradigm of nutritional theory should be expanded with a number of concepts that characterize such information exchange.

Obviously, through complex management of factors that are practically not taken into account at present, it is possible to influence with the desired effect the physiological and biochemical processes in the body. Such factors include not only chemical (biologically active substances, phytohormones, neuropeptides, some amino acids, fatty acids, etc.) and physical (temperature, humidity, particle size and stiffness) parameters of the food itself, but also the frequency of feeding, phased feeding, sound and light effects. In aggregate, we propose to call them the informational component of food, without the rationing of which it is impossible to improve feeding of animals, especially highly productive ones.

From the practical problems, it is possible to limit the scope of the general theory of nutrition to the concept of rationed feeding. In this case, instead of the postulates of theories of balanced nutrition and adequate nutrition [26], we offer the following definition, which introduces the necessary and sufficient fundamental concepts. Normalized nutrition is a process of material and information

exchange between the external environment and the body, which ensures the full-fledged productive life of the latter. The concept of "material and information exchange" means that this process involves both the actual nutrients of the feed and its undigested residues removed from the body, as well as information signals from chemical agents and physical factors associated with food (some amino acids, hormones and hormone-like substances, biologically active substances; food structure, regularity and volume of serving portions), and from factors of non-food nature (insolation, temperature and humidity, acoustic accompaniment of the feeding process).

Information exchange is accompanied by a change in the informational state of the organism. The term "stress" is used to assess the informational state. There are three stages in the development of stress: mobilization of the body's adaptive capabilities (anxiety stage), balanced expenditure of adaptation reserves (resistance stage), and exhaustion stage [28]. Exhaustion is not understood as the expenditure of metabolic resources, but a kind of "fatigue" of the nervous or, rather, the neurohumoral system. Currently, it is customary to assess stress by the effect of a stress factor on the activation of the hypothalamic-pituitary-adrenal axis (HPA axis) [29]. In various animal species, including ruminants, increased activity of the HPA axis is usually observed in connection with acute stress and is aimed at mobilizing the body's resources to overcome the effects of the stress factor. Corticoliberin, a peptide hormone secreted by neuroendocrine cells of the hypothalamus, at the level of the central nervous system, is involved in the regulation of feed intake, as well as in behavioral responses to stress [30, 31]. At the same time, under repeated or long-term exposure to stressors in ruminants, the regulation of the HPA axis is not entirely understood and can be carried out by both the adrenal glands [32] and the pituitary gland [33]. Currently, primary attention is focused on studying the effect of nutrition on the HPA axis reactivity in ruminants in order to optimize the number of animals in the production group, the space for their feeding and rest [34, 35]. In our opinion, these studies are of particular interest in connection with the development of a methodology for assessing the current state of the body.

A significant part of the information flow from the body is directed to the lumen of the gastrointestinal tract and interacts with the microflora that lives there, which, in turn, interacts with the macroorganism, producing certain signaling substances (and quite possibly through other communication channels).

A full-fledged vital activity means the expenditure of energy and metabolites obtained from food to maintain vital functions, physical and intellectual activity, to increase body weight, growth and development, to reproduce (in agricultural animals, also to ensure productivity in breeding goals) for a sufficiently long time without harm to the body.

In the existing norms for human nutrition and feeding of agricultural animals, there is practically no study on rationing the information flow accompanying feeding and digestion. Basically, the information component is partially standardized by medical and veterinary regulations, sanitary standards and requirements for working conditions and human life, as well as zootechnical requirements for the conditions of rearing farm animals. The present approach to rationing can be characterized as deterministic in the sense that, according to it, for the obtaining a certain amount of production, an animal a priori requires certain conditions of rearing, the amount of energy and plastic substances. In this case, neither the individual, nor the adaptive capabilities of the organism are taken into account. Obviously, the primary task in the development of rationed feeding systems should not be the creation of averaged norms of need,

but the development of methods for assessing and predicting the state of the animal's body as the controlled object [36].

Thus, the classical theory of balanced (adequate) nutrition requires correction to incorporate in its paradigm the concepts of the informational component of food and the circulation of information between the environment and the body. Structural orderliness of the evaluated object (e.g., food, chyme, or feces) can be expressed as the product of the average particle size and the dry matter content. The average particle size of the diet in the range studied by us and feed structure are inversely related to the average size and structure of the contents of the gastrointestinal tract and feces. No relationship has been established between the mean particle size of the diet, the structure of food and rumen content, on the one hand, and the duration of chewing or the number of rumen contractions, on the other. An increase in structure did not have a significant effect on the digestibility of the main groups of nutrients in the feed, with some improvement in the conditions for the synthesis of microbial crude protein in the rumen.

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