Physiological Communication

Cellulosolytic Activity of Gastrointestinal Microflora and Energy Metabolism in Yakutian Horses During Winter

R.V. Ivanov[1], A.M. Stepanova[2], U.V. Khompodoeva[1], M.N. Pak[1], I.V. Alferov[1], L.G. Kozlova[1]* and V.V. Sysolyatina[3]

[1]Laboratory of Selection and Breeding of Horses, M.G. Safronov Yakut Scientific Research Institute of Agriculture, The Yakut Scientific Centre of the Siberian Branch of the Russian Academy of Sciences, Yakutsk, Russia

[2]Laboratory of Microbial Preparations, The Yakut Scientific Centre of the Siberian Branch of the Russian Academy of Sciences, M.G. Safronov Yakut scientific research institute of agriculture, Yakutsk, Russia

[3]Cattle Selection and Breeding Laboratory, The Yakut Scientific Centre of the Siberian Branch of the Russian Academy of Sciences, M.G. Safronov Yakut scientific research institute of agriculture, Yakutsk, Russia

[4]Agrotechnological Faculty, Yakutsk State Agricultural Academy, Yakutsk, Russia

ABSTRACT

The article presents a study to improve the technology for winter feeding of horses of the Yakut breed. Feeding technology affects digestion and assimilation of the carbohydrate complex of plant feed since the supply of horses with energy at negative temperatures, the physical and technical properties of wool, and the quality of meat products depend on the absorption of nutrients by the body. The article presents a comparative study of the cellulolytic activity of the microflora of the gastrointestinal tract and energy metabolism of adult horses and foals at the age of 9 months and the efficiency of including the probiotic "Sakhabactisubtil" into the compound starter feed for foals under one year of age. As a result of the research, it has been found that during the winter, when consuming the hay and oat diet, foals at the age of 9 months consume considerably more metabolic energy per kilogram of metabolic weight than mares. A mare used 0.99 MJ of energy per kilogram of metabolic weight (LW+0.75), while foals used 1.39 MJ, which was 40% more. In the experiment to establish the efficiency of using compound starter feed with the probiotic "Sakhabactisubtil", young horses of the experimental groups used the nutrients of the feed better.

KEY WORDS: COMPOUND STARTER FEED, FOALS, HAY AND OAT RATION, MARES, PROBIOTIC "SAKHABACTISUBTIL", YAKUTIAN HORSE.

INTRODUCTION

Yakutian horses are pastured all year round, consuming pasture fodder, with only 10-15% of their annual ration being hay with a small amount of oats. However, for 7 months of the year, horses consume the haylage of winter pasture which represents grassland litter or aftergrass. The chemical composition of the aftergrass is dominated by crude fibre (35%) and nitrogen-free extractive substances (44.7%). Crude fat is 4.2%, crude ash is 7.8%, and only crude protein is 8.8%. With ageing, lignin accumulates in plants, which creates a solid carbohydrate-lignin complex, which reduces the digestibility of fibre and non-natural extractive substances in farm animals. The accumulation of knowledge on the digestibility and assimilation of carbohydrate complex of plant fodder is important to improve the technology of winter feeding of Yakutian horses because it affects the energy supply of horses at subzero temperatures. A horse consumes fodder firstly using a maximum of easily accessible nutrients in the stomach and intestine: soluble proteins, starches, sugars, minerals, vitamins, and in the large intestine, with the help of residual enzymes of the small intestine and microorganisms, utilizes hardly accessible fibre, residues of protein, carbohydrates, and minerals (Novakovskaya, 2015; Khompodoeva et al., 2020; Ivanov et al., 2020).

Article Information:*Corresponding Author: klg_14@mail.ru
Received 15/07/2021 Accepted after revision 28/09/2021
Published: 30th September 2021 Pp- 1309-1317

Available at: https://bbrc.in/
Article DOI: http://dx.doi.org/10.21786/bbrc/14.3.60

The horse's small intestine digests 60 to 70% protein, 65 to 75% soluble carbohydrates, and only 15 to 25% fibre. A large part of the diet, 75-85% fibre, 30-40% protein, and 25-35% soluble carbohydrates, is digested in the large intestine. Fibre digestion is carried out by cellulosolytic bacteria. These processes are as active as rumen digestion in corresponding cattle. According to the currently adopted technology, foals are weaned for sale for meat and for repair at the age of 6-7 months in November at a lower air temperature of 15-20°C, when the carcasses of slaughtered foals are rapidly cooled. In this case, the fatness of foals left for repair is reduced in the first days and the animals are poorly nourished due to the stress of weaning and the unfamiliar hay and oat ration. A slowdown in the rate of growth and development of foals occurs during the whole winter period with an almost complete halt in mid-winter. Meanwhile, it is known in the Yakutian horse breeding practice that foals left on a suckling with mothers without weaning are also growing well in winter. At the same time, foals' growth and development depend on the mother's milk yield (Gabyshev, 2002; Vinokurov, 2012; Sharaskina, 2019).

This peculiarity of foals' growth and development during the first winter shows how important the quality of foals' nutrition is. It seems to us that the cellulosolytic activity of the microflora of the gastrointestinal tract and the energy metabolism of the foals are important as well. At this time, the foals are transitioning from a diet of mother's milk and green pasture grass to a hay and oat diet, i.e. a transition from an easily digestible carbohydrate diet to the digestion of feed with hard-to-digest fibre, with a high content of almost indigestible lignin. We also suggest that in young horses aged 7-11 months, the cellulosolytic activity of the gastrointestinal tract is less active than in adult animals. The important thing in the nutrition of young foals under 1 year of age is energy supply. Both the integument and, most importantly, the subcutaneous and intra-abdominal fat insulate the body less from the cold than in adult animals because the coat in foals is shorter and fat deposits are thinner than in adult animals. Equally important is the duration of feed intake, as it generates heat, defined as a specific dynamic action of food or incremental heat production (Novakovskaya, 2015; Ivanov, 2018; Ivanov et al., 2021).

This heat under conditions of stress from freezing helps to maintain the body temperature of the animal. Based on the results of many years of research, it has been established that bacteria of the genus Bacillus dominate in the microbiocenosis of frozen soils in Yakutia. The bacterial strains B. subtilis TNP-3 and B. subtilis TNP-5 isolated from perennially frozen soils possess a wide range of unique biological properties: pronounced antagonistic action against context-dependent pathogenic and pathogenic microorganisms (bacteria, fungi, and viruses), interferon-inducing activity, immunostimulating effect, antibiotic resistance, ability to stimulate growth and development of beneficial intestinal microflora, in particular, bacteria of Lactobacillus and Bifidobacterium. The unique biologically active properties of strains B. subtilis TNP-3 and B. subtilis TNP-5 have led to the development of the biopreparation "Sakhabactisubtil". The preparation is widely used in northern animal breeding: in respiratory diseases, digestion, reproduction, dysbacteriosis, and mycotoxicosis, also as a part of hygienic and polishing agents, is effective for silage and haying of forages, for rehabilitation of oil-polluted soils, besides, it is a part of inactivated vaccines against horse infectious diseases (Vladimirov et al., 2020; Neustroev et al., 2020b, Neustroev and Tarabukina, 2021).

The studied strains of B. subtilis TNP-3, TNP-5, and their combination were found to possess cellulase activity. According to Russian and foreign scientists, bacteria of the genus Bacillus are characterized by polyenzymatic properties. Bacillus cells include a set of enzymes of different classes, which provides them the ability to exist in different substrates (Laktionov, 2012; Skryabina et al., 2020; Abdelfattah et al., 2015; Murad and Azzaz, 2010). These enzymes can significantly affect feed digestibility and nutrient uptake, improve chemical composition, physical and technical properties of wool, and enhance the quality of meat products. In this respect, our task was to compare the cellulosolytic activity of the microflora of the gastrointestinal tract and energy metabolism of adult horses and young animals at the age of 9 months and to determine the efficiency of including the probiotic "Sakhabactisubtil" into the compound starter feed for young animals up to one year of age.

MATERIAL AND METHODS

The work was carried out at the research and production farm of the Horse Selection and Breeding Laboratory "Olbuordaakh", Amga ulus, of the M.G. Safronov Yakut Scientific Research Institute of Agriculture during the winter of 2019 and 2020. The studies were carried out based on a permit to conduct research work issued by a commission consisting of the head of the nasleg I.V. Semenov and the commission members P.P. Arsentieva and I.V. Kuzmina No. 1-002 dated 16.02.2019. During the experiments, humane research methods were used in relation to animals. Selection and chemical analysis of winter pasture fodder and hay and oat rations were carried out according to the methods of "Modern Biological and Biochemical Research Methods in Zootechnics" (Burtseva and Rudishin, 2014). Laboratory research was carried out on an infrared analyser NIR SCANNER model 4250 in the laboratory of agricultural product processing and biochemical analyses of the Yakut Scientific Research Institute of Agriculture. The formulation of compound starter feed for foals was developed based on establishing energy, nutrient, and mineral deficiencies and following the foals' needs for basic nutrients (Draganov et al., 2010). The experiment on the digestibility of nutrients in the hay and oat ration was conducted on 4 mares of Yakutian breed aged 10 years with approximately the same live weight. The horses consumed 14 kg of hay and 2 kg of oats per day per head. The duration of the preparatory period was 4 days, the accounting period 6 days.

The experiment on the determination of the efficiency of mixed fodders was carried out on 45 foals at the age of 9 months during stationary winter feeding. Three groups of animals were formed, selected on the principle of analogue

pairs by live weight, age, and body type. Differences between the pairs in body weight and age did not exceed 10%. The control group received a diet consisting of 5 kg of hay and 2 kg of oats. In the first experimental group, the oats were replaced by compound starter feed. The second experimental group received compound starter feed with the inclusion of the probiotic "Sakhabactisubtil". The experimental and control groups of animals were kept in separate barns and the duration of the experiment was 60 days. At the beginning and end of the experiments, animals were weighed and blood samples were taken for analysis. At the end of the feeding experiments, tests were carried out on the digestibility of feed nutrients. To conduct digestibility experiments, 6 horses from each group were placed in special barns to collect faeces and record the feed fed. The duration of the preliminary period was 3 days and the accounting period was 6 days. Fodder and faeces sampling and storage were conducted according to the methods of "Modern Biological and Biochemical Research Methods in Zootechnics" (Burtseva and Rudishin, 2014). Energy value of fodder – by the regression equation of the All-Russian Research Institute of Horse Breeding (Kosharov et al., 1983).

ME = 19.46dP+35.43dF+15.95dFb+15.95dNfES, where
ME – metabolic energy of fodder (MJ)
dP – digestible protein, g
dF – digestible fat, g
dFb – digestible fibre, g
dNfES – digestible nitrogen-free extractive substances, g

The content of total energy in diets was calculated according to the equations developed by L.K. Ernst Federal Research Center for Animal Husbandry and Institute of Animal Physiology, Biochemistry and Nutrition:

TE = 24.24cP+38.87cF+18.39cFb+17.14cNfES;
DE = 23.93dP+32.66dF+18.5dFb+17dNfES;

where cP, cF, cFb, cNfES are "crude" nutrients, g; dP, dF, dFb, dNfES are digestible nutrients, g (Nadalyak et al., 1986; Shcheglov, 1991).

Biochemical analyses of fodder, faeces, blood were carried out in the biochemistry laboratory of the M.G. Safronov Yakut Scientific Research Institute of Agriculture. The preparation Sakhabactisubtil was developed from an equal combination of strains of *Bacillus subtilis* TNP-3 and *Bacillus subtilis* TNP-5 with B. subtilis content of 5x109 CFU in 1 ml. The strains of *Bacillus subtilis* TNP-3 and *Bacillus subtilis* TNP-5 were extracted from frozen soils of Yakutia and deposited in the All-Russian collection of microorganisms used in animal husbandry and veterinary medicine (The Russian State Center for Animal Feed and Drug Standardization and Quality, Moscow) (Vladimirov et al., 2020). The drug was given at a dose of 50x109 CFU/ml at the rate of 10 ml per 1 head daily in combination with hay and mixed fodder for 10 days. The preparation Sakhabactisubtil was manufactured by the Scientific and Production Center HOTU-BACT according to TU 9384-003-00670203-06 and was used according to the instructions approved by the Rosselkhoznadzor on 06.07.2012.

Samples of faeces from foals for microbiological studies were taken from three groups – before the experiment and after the experiment. Microbiological studies were carried out in the laboratory for the development of microbial preparations at M.G. Safronov Yakut Scientific Research Institute of Agriculture – Division of Federal Research Centre "The Yakut Scientific Centre of the Siberian Branch of the Russian Academy of Sciences. The following media were used to study the intestinal microbiota of young horses and for the quantitative accounting of isolation and quantification of bacteria: lactobacagar – for lactic acid microorganisms, bifidum medium – for bifidobacteria, azide medium – for enterococci, MAP (after heating to 85ºC for 15 minutes) – for spore-forming aerobic bacteria, Endo – for Escherichia, Baird-Parker – for staphylococci, medium with bromothymol blue – for yersinia, Chapek – for microscopic fungi. Microbial growth was recorded after 18, 24, and 48 hours for bacteria and 5 days for fungi. The number of microorganisms was determined in colony-forming units (CFU) per 1 g. Genus and species identification of microorganisms was carried out according to the "Handbook on microbiological and virological research methods" (1982), "Identification of zoopathogenic microorganisms" (1995), and "Bergi's bacteria identifier" (1997). The main numerical data obtained in the studies were processed by a biometric method using the Microsoft Excel computer program.

RESULTS AND DISCUSSION

Table 1 compares the digestibility of the nutrients in the hay and oats diet in mare and foal experiments.

Table 1. Comparison of the digestibility of nutrients in the hay and oat diet of mares and foals, %

Indicator	Group Mares	Foals
Dry substance	64.04 ± 1.71	63.1±0.06
Organic substance	66.8 ± 4.41	68.2±0.17
Crude protein	69.2 ± 0.17**	73.0±0.08**
Crude fat	60.9 ± 0.48*	62.01±0.32*
Crude fibre	52.5 ± 0.71**	49.5±0.25**
NfES	72.7 ± 1.42	70.14±0.60

Note: * – P ≥ 0.95; **- P ≥ 0.99; *** – P ≥ 0.999

The table shows that digestibility ratios for all components were higher in foals, except crude fibre and nitrogen-free extractable substances. Comparatively high ratios of crude protein and fat in foals, probably, were connected with the fact that their diet contained relatively more oats than the diet of mares (foals had 2 kg of oats in 5 kg of hay, mares had 2 kg of oats in 14 kg of hay). The chemical composition of oats in both experiments was practically the same. Epy composition of hay in the experiments is given in Table 2.

Table 2. Chemical content of hay in experiments on mares and foals in winter (percentage of dry substance)

Indicator	Mares	Foals
Indicator	Mares	Foals
Water	5.45 ± 0.13	5.26±0.11
Percentage per substance:		
Crude protein	14.42 ± 0.61**	17.68±0.09**
Crude fat	3.16 ± 0.64	2.72±0.06
Crude fibre	32.85 ± 0.32**	37.02±0.50**
NfES	27.24 ± 0.84*	30.32±0.12*
Crude ash	6.72 ± 0.18	7.00±0.09

Note: * – P ≥ 0.95; **- P ≥ 0.99;

Table 2 shows that the hay consumed by the foals had more crude protein, fibre, and nitrogen-free extractive substances. This is why the digestibility of protein was higher, while fibre and NfES were lower in the foals. The carbohydrate-lignin complex of foals' hay was less accessible to the cellulosolytic bacteria in the colostrum of foals compared to mares. The relatively low digestibility of the carbohydrate part of the diet can also be explained by the insufficient establishment of cellulosolytic activity of the gastrointestinal tract microflora of foals at 9 months of age, or simply by the insufficient number of cellulosolytic bacteria in the large intestine of foals compared to the intestines of adult horses. In the conditions of the Yakut winter at minus 45-55 degrees C, the knowledge of the amount of metabolic energy for life sustenance and super-support energy, not only theoretical but also practical, is of great importance.

Table 3. Energy consumption and utilisation by mares and foals, MJ

Indicator	Group	
	Mares	Foals
Energy consumed	170.24 ± 5.12	135.2 ± 1.12
Digestible	107.48 ± 2.41	82.1 ± 0.41
Faeces	62.75 ± 2.84	53.1+0.64
Urine and methane	11.16 ± 9.32	9.6+0.35
Metabolic	96.31 ± 1.17	72.5 ± 1.15
Incl. for life sustenance	50.95 ± 1.41	42.25+0.83
Super-support energy	45.36 ± 0.71	30.25+
The concentration of metabolic energy in 1 kg of dry substance	9.0 ± 0.12	9.56+0.24
Total energy metabolism, %	56.57	53.5

The quantitative characteristic of energy of urine and methane was determined according to the reference book "Farm animals. Physiological and biochemical parameters of the organism" (Reshetov, 2002). The live weight of the mares was on average 425kg, the foals were 208kg, i.e. the mares weighed more than twice as much as the foals. However, metabolic energy intake was only 33% higher. This can probably be explained by the fact that the foals also had a comparatively higher energy expenditure to sustain life. According to our calculations, the difference in metabolic energy expenditure for life sustenance in mares and foals was only 20%. This can be explained by the fact that foals expend more sustainable energy in winter than adult animals. This is due to the fact that they seem to have worse thermal insulation than adult animals. Foals have shorter coats, and their skin and subcutaneous fat are thinner than in mares. They also have much less body fat and internal fat in the abdomen. Therefore, energy losses in foals are higher than in mares, and there is more expenditure on heat production as well. It should be noted that the expenditure of super-support energy already differed by 50%, which is probably due to the fact that at this time (March), intense fetal growth begins in mares, while the growth and development of foals are almost stopped. Per kilogram of metabolic weight (LW+0.75), mares use 0.99 MJ of energy, while foals use 1.39 MJ, or 40% more.

Table 4. Deficiency of energy, protein, fibre, macro-microelements, and vitamins in the hay and oat diet of Yakutian foals

Indicator	Fodder			
	Norm	Oat	Hay	Deficit
Crude protein, g	912.8	216	425	271.8
Fibre, g	1,152.1	194	1,214	+
Metabolic energy, MJ	60.7	18.4	30.05	12.25
Calcium, g	47.6	3.0	36.0	8.6
Phosphorus, g	34.2	6.8	12.5	14.9
Magnesium, g	9.07	2.4	10.5	+
Iron, g	680.4	82.0	49.1	549.3
Copper, g	60.78	9.8	10.5	40.48
Zinc, g	215.4	45	91	79.4
Cobalt, mg	3.62	0.14	0.95	2.53
Manganese, mg	272.1	113	280	+
Iodine, mg	4.08	0.2	1.45	2.43
Selenium, mg	2.04	-	0.095	1.945
Carotene, mg	68.04	-	12.5	55.54
Vitamins: A, 1000 IU	18.14	2.0	-	16.14
D, 1000 IU	1.81	-	-	1.81
E, mg	204.2	26.0	96.0	82.2
B1, mg	19.9	14.6	6.5	+
B2, mg	19.95	2.2	3.5	14.25
B3, mg	33.56	26.0	6.0	1.56
B4, mg	453.6	1.8	300.0	151.8
B5, mg	6.8	0.26	0.8	5.74
B6, mg	9.9	-	-	9.9
B12, µg	40.8	-	-	40.8
BC, mg	13.6	-	-	13.6
H, mg	453.6	-	-	453.6
C, mg	450.6	-	-	450.6

Table 5. The formula of compound starter feed for young horses up to one year of age

Indicator	Compound starter feed	
	%	RUB/kg
Oats	30	5.25
Barley	7.0	1.31
Wheat bran	14	2.8
Soybean grist (lysine source)	7.0	3.5
Dry hop pellets	31.5	1.57
Sunflower seed cake	8.0	2.48
Kempendyay salt	0.3	0.075
Felucene with biotin, Se, Mg	1.5	1.05
Premix for horses, Classic	0.69	2.34
Probiotic, ml	0.01	0.35
Total	100	20.72

It seems to us that one of the reasons why the growth and development of foals after weaning at the age of 6-7 months is reduced is that at this time, the diet is changing from a milk ration combined with green grass to hay and oats. The microbiota of the gastrointestinal tract must be adapted to digest fodder with high fibre content. The gastrointestinal tract of foals, including the large intestine where fibre digestion by cellulosolytic bacteria occurs, is small compared to that of adult horses. Apparently, for this reason, the fibre digestion of the hay and oat ration is not high enough, resulting in a low energy intake, which leads to a delay in the growth and development of the foals in the first wintering period. Therefore, we set ourselves the task of developing a formula for compound starter feed to increase the growth and development of foals up to a year old in winter stationary feeding. Besides, considering probiotic, cellulosolytic activity, the preparation "Sakhabactisubtil" was included in the recipe of the second experimental group of foals. To compose the formula for compound starter feed, the deficit in energy, protein, fibre, minerals, and vitamins in young horses under 1 year of age was calculated (Table 4).

Table 4 shows that when consuming 7.58-7.64 kg of dry substance of the hay and oat ration, young horses up to one year of age have a deficit of almost all basic nutrients, macro- and microelements, and vitamins, except for crude fibre, magnesium, and manganese. To compensate for the deficiency of the main nutrients, macro-, and microelements for young horses up to one year old, we worked out a formula of compound starter feed (Table 5). Weighing of foals at the beginning and the end of the experiment showed that during the period of the experiment, the live weight increased from 201.44 kg to 217.0 kg (+15.56 kg) in animals of the first experimental group, the increase was from 200.5 kg to 220.3 kg (+19.8 kg) in animals of the second experimental group, and from 202.53 kg to 208.46 kg (+5.93 kg) in the control group. The difference in live weight between the animals of the control and experimental groups at the time of setting of the experiment was minimal and amounted to 1090 and 2030 grams, but at the end of the experiment, it was 8.54 and 11.8 kg or an absolute gain in the animals of experimental groups at the end of the experiment was 2.6 and 3.3 times more than the control group (Table 6).

Table 6. Live weight of young Yakutian horses before one year of age

Indicator	Group Control		Experimental I		Experimental II	
	Beginning	End	Beginning	End	Beginning	End
Live weight, kg	202.53±2.67	208.46±2.46	201.44±2.96	217.0±3.88**	200.5±1.78	220.3±3.02**
Growth rate	2.93	7.72	9.88			
Absolute growth rate	5.93	15.56	19.8			
**P ≥ 0.999						

Table 7. Measurements of young horses under 1 year of age for the period of the experiment

Indicator	Group Control		Experimental I		Experimental II	
	Beginning	End	Beginning	End	Beginning	End
Height at withers, cm	123.5±2.32	123.7±2.45	121.9±2.3	126.1±2.43**	125.3±2.75	128.7±2.82**
Oblique length of torso, cm	123.7±3.80	123.9±4.18	121.8±2.71	124.9±2.88*	123.7±3.74	132.5±4.76*
Chest circumference, cm	143.1±3.98	143.3±1.58	140.4±4.45	143.9±4.55**	139.0±3.29	145.1±3.50**
*P ≥ 0.95; **P ≥ 0.999						

The average daily gain of the young Yakutian horses during the period of the experiment, in animals of experimental groups was 280 and 360 g, in the control group – 108 g. Thus, a reliable increase in live weight was observed in the animals of both experimental groups 7,17 and 8,99 % (P ≥ 0,999) respectively, the control group animals showed a non-significant difference in growth (2,84 %). Table 7 represents the data of the experiment on the measurements of young horses for the period of the experiment.

Table 8. Amount of nutrients consumed by young animals, g (average per animal per day)

Indicator	Group		
	Control	Experimental I*	Experimental II**
Dry substance	7,584.6±31.39	7,621.89,±44.39	7,638.29±47.42
Organic substance	6,789.94±28.30	6,903.91±41.68	6,901.89±42.95
Crude protein	1,409.34±17.60	1,303.96±41.68	1,315.30±17.60
Crude fat	230.77±7.10	266.15±41.68	263.61±10.40
Crude fibre	3,096.533±88.70	2,392.13±41.68	2,383.27±22.70
NfES	2,053.3±92.30	2,941.67±41.68	2,939.71±52.70

* without a probiotic culture
** with a probiotic culture

Table 9. Digestibility coefficients of the main nutrients in the diet of young animals during the experiment period, %

Indicator	Group		
	Control	Experimental I*	Experimental II**
Dry substance	63.1±0.06	63.7±0.±0.19	66.5±0.08*
Organic substance	68.2±0.17	68.8±0.11	70.1±0.20*
Crude protein	73.0±0.08	74.1±0.08	74.8±0.20
Crude fat	62.01±0.32	63.56±0.26	65.64±0.50
Crude fibre	49.5±0.25	50.3±0.25	52.66±0.28*
NfES	70.14±0.60	72.1±0.60	73.48±1.10*

*P ≥ 0.95

It was found that the height at withers in young animals of the first experimental group was increased by 3.3 % and was 126.1±2.43 cm (P ≥ 0.999), chest circumference was increased by 2.4 % and was 143.9±4.55 cm (P ≥ 0.95), in young animals of the second experimental group the height at withers increased by 2.6% to 128.7±2.82 cm (P ≥ 0.999), chest circumference increased by 4.2% to 145.1±3.50 cm (P ≥ 0.95), in the control group – 123.7±2.45 cm and 143.3±1.58 cm respectively. When studying the effect of the feed factor on the animal's organism, the assessment and analysis of the indices of digestibility and use of nutrients in the feed is of great importance. We calculated the amount of nutrients and energy ingested by the experimental animals during the day based on the chemical composition and the amount of feed that was eaten. Table 8 shows that the amount of dry substance, organic substance, and crude protein consumed in all groups was approximately equal. The daily amount of nutrients ingested with the diet is not fully absorbed by the animals, and a certain part of them is excreted with the faeces. The difference between the amount of nutrients entering the gastrointestinal tract of the animals and the amount excreted in the faeces characterizes the amount of digested nutrients.

The data we obtained during the scientific and economic experiment show that the greatest amount of nutrients in the diets was digested by the animals fed with compound feed with the probiotic culture. At the same time, young animals of the first experimental group surpassed their control group counterparts by 0.6% in the digestion of dry substance, organic substance also by 0.6%, protein by 1.1%, fat by 1.55%, and fibre by 0.8%. The analyzed data show superiority in digestibility of young animals of experimental groups over their counterparts from the control group. As for the youngsters of the second experimental group that consumed compound feed with the probiotic culture,

they surpassed their counterparts of the control and first experimental group on all indicators, namely, by 2.8% on dry matter of the first experimental group and by 3.4% of the control, organic matter by 1.3% of the first experimental group and by 1.9% of the control, protein by 0.7% and 1.8% respectively, fat by 2.08% and by 3.63%, and fibre by 2.36% and 3.16% – the control group.

A relatively high intake of nutrients throughout the experiment provided the young horses of the experimental groups with higher coefficients of digestibility of the main nutrients. Remarkably, young horses of the control group were inferior to their counterparts of experimental group I in terms of NfES digestibility coefficient by 1.96% ($P \geq 0.95$), and those of experimental group II by 3.34%. Inclusion of compound feed into the winter ration at stationary feeding of foals had a positive effect on feed intake, digestibility and assimilability of nutrients in the ration, while compound feed with the addition of the probiotic "Sakhabactisubtil" promotes even more successful assimilation of fodder in the ration of horses.

When studying the metabolism of substances and energy in the body, as well as evaluating the nutritional value of feed and rationing of animals, the following types of energy are distinguished: total, digestible, metabolic (or physiological), the energy of heat production, and energy released during production. The transformation of feed energy into animal products is significantly affected by the level of feeding, the structure of the ration, the concentration of energy per unit of dry substance, as well as the balance of the ration on mineral elements and biologically active substances (Kalashnikov et al, 2011, Ivanov et al, 2020, Ivanov, et al, 2021). The experimental data we obtained in the physiological experiment testify to the positive effect of compound starter feed when including it instead of oats in the oat and hay ration during winter stationary feeding of young horses under one year of age on energy intake and utilization.

Table 10. Energy intake and utilization by young Yakutian horses in winter experiments, MJ

Indicator	Group		
	Control	Experimental I	Experimental II
Energy: Total	135.2 ± 1.12	136.3 ± 0.17	136.3± 2.14
digestible	82.1 ± 0.41	86.9± 0.35*	89.1 ± 0.51*
faeces	53.1±0.64	49.4±0.72	47.2±0.54
urine and methane	9.6±0.35	9.1±0.43	9.4±0.48
metabolic	72.5± 1.15	77.8± 1.25*	79.7 ± 1.19*
Incl. life-sustaining energy	42.25±0.83	42.59±1.09	42.6±0.96
Super-support energy	30.25	35.21	37.1
The concentration of metabolic energy in 1 kg of dry substance	9.56±0.24	10.21±0.11	10.43±0.23
Total energy metabolism, %	53.5	57.0	58.4
*$P \geq 0.95$			

The amount of total energy consumed by young animals of experimental and control groups was comparatively equal, the indicators of experimental group I and II exceeded the control group counterparts in this indicator by only 1.1 MJ (0.80%). At the same time, the young animals of experimental group I exceeded their control group counterparts in digestible energy by – 5.52% and metabolizable energy by – 6.81%. Experimental group II outperformed the control group in digestible energy by – 7.85%, metabolizable energy by – 9.03%. As a result, the metabolism total energy in young animals of experimental group I that consumed compound starter feed was higher – by 6.14% than in the control group counterparts. As for the young animals of the experimental group II, which consumed compound feed with probiotic culture, they surpassed their counterparts from the control group by 8.39%. The analyzed data show that the metabolism rate of the total energy of the experimental group II, which consumed the compound feed with the probiotic culture, is the highest. Thus, the obtained data indicate that feeding young animals with compound feed with the addition of the probiotic culture "Sakhabactisubtil" in the spring-winter period contributed to a significant increase in the productive use of energy (Koilybaeva et al., 2018; Neustroev et al., 2020a; Vinokurova et al., 2021).

Currently, spore-forming bacteria of the genus Bacillus are widely used as probiotic additives, as well as feed additives for farm animals (Koilybaeva et al., 2018; Vinokurova et al., 2021, Neustroev et al., 2020b). Such qualities of bacilli as high and varied biological activity, the ability to survive in the gastrointestinal tract of animals, and the thermal resistance of spores make these bacteria attractive as probiotics and appealing for further search for active strains and the creation of new highly effective drugs. As a result of the studies carried out by Neustroev, it was found that when using the drug Sakhabactisubtil (single intragastric administration to mice of the DM 1 line), mortality or

any signs of intoxication in animals were absent when the drug was administered to them in the maximum allowable volumes for animals of this type. Therefore, the drug does not have a potential toxic effect (Neustroev et al., 2020a).

CONCLUSION

Experimental data show that in winter when consuming a hay and oats diet, foals at 9 months of age consume significantly more metabolic energy per kilogram of metabolic weight than mares. Per kilo of metabolic weight (LW+0.75) mares have 0.99 MJ of energy consumption, while foals have 1.39 MJ, which is 40% more. In the experiment on establishing the efficiency of using the compound feed starter with the probiotic "Sakhabactisubtil", young horses of the experimental groups used the nutrients in the feed better. Reliably high coefficients of digestibility were observed in the experimental group II on dry matter by 5.1% (66.5±0.08%), on organic matter by 2.7% (70.1±0.20%), on crude fibre by 6.0% (52.66±0.28%), and on BEB by 4.5% (73.48±1.10%). Young animals of the experimental groups I and II outperformed their counterparts in digestible energy by – 5.52 and 7.85% (136.3 ± 0.17 and 136.3 ± 2.14 MJ), metabolism energy by – 6.81 and 9.03% (77.8 ± 1.25 and 79.7 ± 1.19 MJ), respectively ($P \geq 0.95$). The metabolism of total energy in young animals of experimental groups I and II was higher, by – 6.14 and 8.39% than in the control group counterparts. The obtained data indicate that replacement of oats in the hay and oat ration during winter stationary feeding of young animals with the compound starter feed offered by us contributed to a significant increase in the growth and live weight of foals of experimental groups due to a significant increase in the productive use of energy.

REFERENCES

Abdelfattah MS et al. (2015). Effects of cellulase and xylanase enzymes mixed with increasing doses of Salix babylonica extract on In vitro rumen gas production kinetics of a mixture of corn silage with concentrate. Journal of Integrative Agriculture No 14(1) Pages 131–139

Birger MO (1982). A handbook on microbiological and virological investigation methods Moscow Meditsina

Burtseva SV and Rudishin OYu (2014). Modern biological and biochemical research methods in zootechnology. Barnaul. Publishing house of AGAU.

Draganova IF (2010). Animal feeding. In 2 volumes.

Gabyshev MF, Kazanskii AV (2002). Fodder grasses of Yakutia: The characteristics of the chemical composition and nutritional value of fodder grasses of the Yakut ASSR. Yakutsk

Hoult J et al. (1997). Bergey's Bacterial Identifier. Moscow. Mir.

Ivanov RV, Alferov IV (2020). Consumption and digestibility of grazing feed nutrients by horses of the Yakut breed in free grazing. Agrarian Russia No 7 Pages 32-35.

Ivanov RV, Khompodoeva UV, Alferov IV, Pak MN, Shakhurdin DN (2021). The effectiveness of using a starter compound feed for young horses of the Yakut breed in the winter. Kormoproizvodstvo No 3 Pages 38-48.

Ivanov RV, Permyakova PF (2018). Scientific basis for improving the technology of feeding and keeping horses of the Yakut breed. Ministry of Science and Higher Education of the Russian Federation (Ministry of Education and Science of Russia), Yakutsk Research Institute of Agriculture named after M.G. Safronov. Novosibirsk. Volume Part II Experiments on adult horses

Kalashnikov BB, .Dpaganov IF, Memedeikin BG (2011). Horse feeding: a textbook. Moscow GEOTAP – Media.

Khompodoeva UV, Ivanov RV, Pak MN, Nikolaev NA, Shahurdin DN, Alferov IV (2020). Energy metabolism in horses of the Yakut breed in the winter when using feed additives in places where they are young. Kormoproizvodstvo. No 8 Pages 43-48.

Koilybaeva MK, Ustenova GO, Batyrbaeva DZh, Alibaeva ZhS, Mustafina KK (2018). Prospects for the use of probiotics based on bacteria of the genus Bacillus. Bulletin of KazNMU No 4-2018 Pages 181-184.

Kosharov AN, Ugadchikov ST, Memedeikin VG (1983). Theoretical justification and recommendations on the use of new feeding norms for horses. Improvement of horse breed selection. VNIIK.

Laktionov KS (2012). A study of cellulolytic activity of cecal bacteria in rabbits by In vitro method. Bulletin of the Orel State Agrarian University No 1 Pages 122-124.

Murad HA, Azzaz HH (2010). Cellulase and Dairy Animal Feeding. Biotechnology. No 9 Pages 238-256. https://dx.doi.org/10.3923/biotech.2010.238.256

Neustroev MP, Murashev AN, Bondarenko DA, Stepanova AM, Tarabukina NP (2020a). Determination of the maximum tolerant dose of the drug Sakhabactisubtil on CD-1 mice. Russian journal of problems of veterinary sanitation, hygiene and ecology No 2(34) Pages 240-244.

Neustroev MP, Tarabukina NP (2021). Problems and prospects for the development of biological preparations in Yakutia. in Proceedings of the Scientific and educational environment as the basis for the development of the agro-industrial complex of the Arctic territories. Pages 142-148.

Neustroev MP, Tarabukina NP, Stepanova AM, Neustroev MM (2020b). Development and production of biological preparations using biological resources of the Arctic and subarctic. In the collection: Agricultural science - agricultural production in Siberia, Mongolia, Kazakhstan, Belarus and Bulgaria. Collection of reports XXIII Intern. scientific and technical Conference, Minsk, 317-321.

Novakovskaya VYu (2015). Changes in the carbohydrate-lignin complex of feed under the influence of enzyme preparations. Bulletin of Altai State Agar University No

10 (132) Pages 66-70.

Reshetov VB (2002). Farm animals. Physiological and biochemical parameters of the organism. VNIIFBiPSZh. Borovsk.

Sharaskina OG (2019). Modern problems of rationed feeding of horses. In the collection: Scientific support for the development of the agro-industrial complex in the context of import substitution. collection of scientific papers based on the materials of the international scientific-practical conference, dedicated to the 115th anniversary of the St. Petersburg State Agrarian University. Pages 289-292.

Shcheglov VV (1991). Indirect methods of determining metabolic energy in feed and rations. Zootechnia No 12 Pages15

Sidorov MA, Skorodumov DI, Fedorov VB (1995). Identifier of zoopathogenic microorganisms. Moscow. Kolos.

Skryabina MP, Stepanova AM, Tarabukina NP, Neustroev MP (2020). The determination of fermentative activity of strains of *Bacillus subtilis* bacteria TNP-3 and TNP5 isolated from frozen soils of Yakutia. Problems of Sanitary, Hygiene and Ecology No 1(33). 73-79 pp., DOI: 10.36871/vet.san.hyg.ecol.202001011

Vinokurov NT (2012). Improving the technology of keeping horses of the Yan type of the Yakut breed in the north-east of Yakutia: Oymyakonsky region: dissertation abstract for the degree of candidate of agricultural sciences: 06.02.10. Yakut state agricultural academy. Yakutsk

Vinokurova DA, Neustroev MP, Tarabukina NP (2021). The use of probiotic from bacterial strains *Bacillus subtilis* tnp-3 and Basillus subtilis tnp-5 for the prevention and treatment of diseases of the Yakut huskies. Proceedings of the Scientific and educational environment as the basis for the development of the agro-industrial complex of the Arctic territories. Pages 210-214.

Vladimirov LN, Neustroev MP, Tarabukina NP (2020). Arctic strains of *Bacillus subtilis* in modern microbiotechnology. Veterinariya i kormlenie No 2 Pages 17-20. https://dx.doi.org/10.30917,ATT-VK-1814-9588-2020-2b