Building a model for predicting digestive enzymes activity depending on lipid composition of diet

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Abstract. Animal nutrition is an important aspect in optimizing the production process of meat and dairy farming. In the process of feeding animals, various digestive enzymes are released, which contribute to the proper functioning of the animal's body. Biochemical blood indicators can be an indicator of health. They can characterize the level of animals’ adaptation to various stressful factors including the specific conditions of animal feeding. Research results show that a high correlation between the digestive enzymes of the pancreas and biochemical parameters of calves’ blood was shown with regards to the time factor and fat component when they were injected with vegetable fats. The dependent relationship in the experimental groups was as follows: sunflower oil (+) uric and acid lipase ($r=0.57$), total bilirubin and protease ($r=0.51$), glucose and lipase ($r=0.68$), protease and total protein ($r=0.59$), protease and albumin ($r=0.51$), protease and total bilirubin ($r=0.63$), protease–phosphorus ($r=–0.55$), respectively. (–) AST amylase ($r=–0.54$), ALT lipase ($r=–0.52$), amylase and total bilirubin ($r=–0.68$), amylase and iron ($r=–0.62$). Palm oil (+) total protein and lipase ($r=0.58$), glucose and protease ($r=0.51$), AST protease ($r=0.54$), iron amylase ($r=0.57$), calcium and lipase ($r=0.63$), negative gamma–GT and lipase ($r=–0.53$). Soybean oil (+) protease and creatinine ($r=0.50$). (–) urea lipase ($r=–0.52$), LDH protease ($r=–0.75$), iron and protease ($r=–0.58$), amylase–iron ($r=–0.65$) amylase–g–GT ($r=–0.64$), amylase–ALT ($r=0.62$). Flaxseed oil (+) albumin protease ($r=0.53$), ALT amylase ($r=0.627$), ALT protease ($r=0.56$), ALT lipase ($r=0.52$), cholesterol amylase ($r=0.55$), phosphorus lipase ($r=0.523$). (–) gamma–GT protease ($r=–0.54$), glucose amylase ($r=–0.63$), glucose protease ($r=–0.52$). Thus, a pronounced positive relationship was observed over a period of time in sunflower (30–60 minutes), palm (60–90 minutes) and linseed (0–30 minutes) oils. Negative effects can be seen when using a diet supplemented with soybean oil. The developed mathematical models make it possible to predict the secretory function of the pancreas and the body’s response to various types of fats, with a database of biochemical parameters and the enzymatic activity of the pancreas.

1. Introduction
Digestion in cattle occurs more intensively than in animals with a single-chamber stomach. The fat component along with proteins and carbohydrates is the key and most energy-intensive compound for animal nutrition. Under the influence of gastrointestinal juice and microbiota containing various enzymes, substances entering the body begin to break down into components [1]

The major role in the life of any organism is assigned to enzymes. Impaired metabolism is determined by impaired synthesis of enzymes. They are actively involved in the process of digesting food, central nervous system functioning and new cells growth stimulation. By their nature, enzymes
are proteins designed to accelerate various biochemical reactions of the body. They have strict specificity, initiate and complete metabolic processes [2–4].

A biochemical analysis of animal blood shows changes in the functioning of the organs of the whole organism, and also helps to identify pathologies [5–6]. Proteins, fats, carbohydrates and minerals breakdown is the process in which enzymes are principle active ingredients. Content of these substances is characterized by constancy, and its change may have informational value in diagnosing diseases [7–8].

Currently, there is a fairly large number of works devoted to the use of fat additives in cattle feeding with regards to the developed nutritional standards and methods for preparing fat components before feeding. Modern biochemistry technologies enable to study a huge number of indicators, but it is not always possible to implement them in a large number of livestock. For this purpose, biochemical parameters of the models aimed to calculate and predict diseases in animals were developed [9–10].

The present work pays special attention to the relationship between the activity of digestive enzymes and biochemical blood parameters depending on the lipids introduced into animal feed and the development of mathematical models.

2. Materials and methods

2.1. Animals and feeding

The studies were performed in the laboratory of biological tests and examinations of the Federal State Budget Scientific Institution of the Federal Scientific Center for Biological Systems and Agricultural Technologies of the Russian Academy of Sciences on the Kazakh Whiteheaded calves (n=3) aged 9 months with an average weight of 215–220 kg.

Animal services and experimental studies were performed in accordance with the instructions and recommendations of Russian Regulations, 1987 (Order No. 755 on December 8th, 1977 the USSR Ministry of Health) and “The Guide for Care and Use of Laboratory Animals (National Academy Press Washington, DC 1996)”. Calves were kept in a specialized cage with free access to water and feeding.

The animals were fed with regards to the main diet compiled with the NRC recommendations (www.nap.edu/catalog/9791.html) and included the following: mixed grass (5 kg), a mixture of concentrates (2 kg), corn silage (1.5 kg), molasses (0.1 kg), fine salt (0.04 kg), vitamin and mineral premix (0.06 kg of elements with the following concentrations per 1 kg: 48 mg of Mn; 36 mg of Zn; 60 mg of Fe; 10 mg of Cu; 0.24 mg of Se; 0.12 mg of Co; vitamin content per kg of concentrate were as follows: 2640 M of vitamin A (VA); 302 IU of vitamin D (VD); 17 mg vitamin E of (VE). Nutrient Requirements of Beef Cattle: Seventh Revised Edition: Update 2000 Subcommittee on Beef Cattle Nutrition, Committee on Animal Nutrition, National Research Council. This PDF is available from the National Academies Press at: http://www.nap.edu/catalog/9791.html

The control calves received the main diet throughout the experiment. The diet of calves of the 1st experimental group included sunflower oil (primary cold pressed, premium), the 2nd experimental group received palm oil (GOST 31647-2012), the 3rd experimental group received soybean oil (STO 40490379-001-2015, TR TS 024/2011), and the 4th group received linseed oil. Vegetable oil was introduced in an amount of 3 % of the dry matter to the grain portion of the diet.

2.2. Observation, estimated indicators

Pancreatic juice was collected from fistulated (5) calves at the age of 9 months with an average weight of 210–220 kg. The experiment was carried out in triplicate using the Latin square 4×4 in the laboratory of biological tests and examinations of the Federal Scientific Center for Biological Systems of the Russian Academy of Sciences. The animals were fed with regards to the main diet, compiled with regards to the recommendations of NRC (1996) [11].

Amylase activity was measured by Smith Roy in the modification aimed to determine the high activity of the enzyme according to Anoson. Proteases was measured by hydrolysis of purified casein according to Hammersten with calorimetric control (wavelength 450 nm), and lipase was measured on
a CS-T240 automatic biochemical analyzer ("Dirui Industrial Co., Ltd", China) using commercial biochemical kits DiaVetTest for veterinary medicine (Russia).

Blood for biochemical parameters was taken from a jugular vein into vacuum tubes with a coagulation activator (thrombin) and evaluated on an automated analyzer CS-T240 (DIRUI Industrial Co., Ltd, China) with commercial veterinary kits (DIAKON-DS CJSC, Russia). Based on the generated database, correlation analysis was carried out for individual diets. The obtained results of the correlation analysis suggest the existence of correlation relationships at a 5% significance level (with a coefficient \( r > 0.5 \)) between the activity of enzymes in pancreatic juice, chyme and the chemical composition of the diet.

Correlation relationships were calculated by the Spearman correlation coefficient. The mathematical model was based on multiple regression dependence obtained by applying the least squares method. In addition, the influence of the time component was singled out separately in the form of a trend model followed by analytical inclusion in the final mathematical model.

2.3. Statistical manipulation

Statistical analysis was performed using ANOVA techniques (software package Statistica 10.0, StatSoft Inc, USA) and Microsoft Excel. The significance of differences in the compared indicators was determined by the Students \( t \)-test. Valid values were considered at \( p < 0.05 \). Data were presented as the mean (\( M \)) and standard error of the mean (\( m \)). Normality testing was carried out according to Kolmogorov-Smirnov criteria. As a result, the hypothesis of the initial data normal distribution was rejected and the use of nonparametric procedures was recommended.

3. Results

A correlation analysis of the enzymes activity in a pancreas with biochemical blood parameters showed the following results: 30 minutes before feeding, no correlation was found, with the exception of a weak positive correlation between uric acid and lipase (\( r = 0.57 \)); 30 minutes after feeding, the number of correlation dependencies increased as well as the negative average between AST and amylase, ALT and lipase, where \( r = -0.54 \). A positive moderate correlation (\( r = 0.51 \)) was noted between total bilirubin and protease. A negative correlation between \( r = -0.68 \) and \( -0.62 \) was detected between amylase with total bilirubin and iron, respectively, as well, a negative correlation was noted between LDH and protease, where \( r = -0.74 \). A comparison of glucose and lipase indicated a positive relationship, namely, an increase in glucose level will lead to lipase activity increase (\( r = 0.68 \)). One hour after feeding, 4 correlations were revealed between the protease and total protein, albumin, total bilirubin and phosphorus, where \( r = 0.59; r = 0.51; r = 0.63; r = -0.55 \), respectively.

The correlation dependence with including palm oil in the diet of experimental calves revealed single moderate correlation. 30 minutes before feeding the dependence was in the form of a weak connection between the total protein and lipase (\( r = 0.58 \)). 30 minutes after feeding, moderate correlations between glucose and protease were detected (\( r = 0.51 \)).

One hour after feeding, a significantly greater number of correlations were found. In particular, the correlations were found between AST and protease (\( r = 0.54 \)), iron and amylase (\( r = 0.57 \)) as well as between calcium and lipase, the latter being somewhat higher (\( r = 0.63 \)). In addition to the above, a negative relationship between gamma–GT and lipase (\( r = -0.53 \)) was also found.

When soybean oil was added to the main diet of experimental calves, two negative connections between urea and lipase (\( r = -0.52 \)) and LDH and protease (\( r = -0.75 \)) were revealed 30 minutes before feeding. 30 minutes after feeding, one negative correlation was observed between iron and protease (\( r = -0.58 \)). One hour after feeding, there was the correlation between amylase and iron (\( r = -0.65 \)), gamma–GT (\( r = -0.64 \)) and ALT (\( r = 0.62 \)) as well as a weak positive relationship between protease and creatinine (\( r = 0.50 \)).

The correlation dependence upon incorporation of linseed oil in calves’ diet revealed moderate correlations between albumin and protease (\( r = 0.53 \)) 30 minutes before feeding and a negative relationship between gamma–GT and protease (\( r = -0.54 \)) 30 minutes after feeding. An hour after
feeding, the number of correlations, mostly positive ones, increased between ALT and amylase ($r=0.627$), ALT and protease ($r=0.56$), AST and lipase ($r=0.52$) and between cholesterol and amylase ($r=0.55$), as well as between phosphorus and lipase ($r=0.523$). A negative moderate relationship was noted between glucose and amylase as well as between glucose and protease ($r=–0.63$ and $r=–0.52$, respectively).

The correlation analysis shows that the relationship in most of the identified cases is significant, but not high. The identified pairs of relationships are rather scattered, which makes their unambiguous assessment difficult. For optimal estimation of changes in functionally significant parameters, mathematical models construction is necessary.

Data analysis shows that the activity of enzymes can vary heterogeneously with regards to the time factor. Thus, the amount of amylase, regardless of the oil type in the diet, first increases in the pancreas, and then decreases, which can be most clearly seen when using a diet with the addition of soybean oil.

The incorporation of palm oil in the diet has a greater effect on the lipase content in the pancreas, and there is practically no change in lipase over time when using this type of oil in the diet of animals. Protease in the pancreas of calves is also subject to changes depending on the oil consumed. In this case, the use of palm oil in the diet has a significant effect as well. Soybean oil affects the protease content to a much lesser extent.

Next, we will carry out mathematical modeling for each of the considered enzymes, taking into account data on biochemical parameters of blood.

In the resulting models, the following notation is applied:

- $z_1$ is glucose;
- $z_2$ is the total protein;
- $z_3$ is albumin;
- $z_4$ is ALT;
- $z_5$ is AST;
- $z_6$ is total bilirubin;
- $z_7$ is direct bilirubin;
- $z_8$ is cholesterol;
- $z_9$ is triglycerides;
- $z_{10}$ is urea;
- $z_{11}$ is creatinine;
- $z_{12}$ is alkaline phosphatase;
- $z_{13}$ is a-amylase;
- $z_{14}$ is gamma-GT;
- $z_{15}$ is uric acid.

All models were obtained by multiple linear regression, $R^2>0.65$.

To assess the effect of the fatty component, the models were also generalized.

A mathematical model that takes into account the effect of blood biochemical parameters on amylase in the pancreas with regards to the time factor and the fat component is as follows:

$$y = t(3734.6–871.84t)(0.0711+0.919)(–25.047z_1 + 0.083z_2 + 1.047z_3 –0.141z_4 + 0.187z_5 –1.598z_6 + 6.211z_7 –139.236z_8 + 50.099z_9 –1.943z_{10} + 0.11z_{11} –0.011z_{12} –0.066z_{13} + 0.849z_{14} + 1.004z_{15} + 908.978).$$

A mathematical model that takes into account the effect of blood biochemical parameters on lipase in the pancreas with regards to the time factor and the fat component is as follows:

$$y = t(465.4–124.31t)(–0.171+1.374)(23.031z_1 – 1.465z_2 + 2.999z_3 – 3.322z_4 – 0.827z_5 + 9.96z_6 + 66.667z_7 + 396.659z_8 – 339.8z_9 – 158.991z_{10} + 0.718z_{11} + 1.166z_{12} –0.046z_{13} + 1.851z_{14} – 4.216z_{15} + 1288.73).$$

A mathematical model that takes into account the influence of blood biochemical parameters on the protease in the pancreas with regards to the time factor and the fat component is as follows:
\[ y = t(-136.7+38.75t)(-0.3l+1.527)(579.882z_1 + 18.926z_2 - 71.752z_3 - 269.611z_4 - 3.565z_5 + 30.087z_6 - 697.578z_7 + 8453.433z_8 - 1803.863z_9 - 253.673z_{10} + 27.178z_{11} - 0.931z_{12} - 17.803z_{13} - 27.809z_{14} + 8.919z_{15} + 5899.909). \]

Thus, as a result of the analysis and subsequent modeling, a number of mathematical models based on correlation and regression dependences and enabling to evaluate the effect of blood biochemical parameters as well as the fat component and time factor on the presence of enzymes in the pancreas have been obtained. All the obtained models have a high degree of reliability (p<0.05) as well as a high degree of significance (R² > 0.65).

4. Discussion

Blood biochemical indicators are the indicators of the whole organism. They can characterize the level of animals’ adaptation to various stressful factors, including the specific conditions of keeping and feeding animals [12–13].

In the process of food digestion, all substances are broken down into small molecules. These are low-molecular compounds that penetrate an intestinal wall and are absorbed into a bloodstream. A special role is given to trypsin and chymotrypsin, amylase and lipase. As a result of the correlation analysis, a reliable dependence of AST-amylase and ALT-lipase was revealed, where r=−0.54 when incorporating sunflower oil into the diet. A weaker relationship was observed between the total protein and lipase (r=0.58) when palm fat was incorporated into the diet [14–15].

It was found that adding sunflower oil into a diet showed a negative relationship between amylase with total bilirubin and iron, r=−0.68 and −0.62, respectively. As well, a negative relationship was noted between LDH and protease, where r=−0.74. The results of the study [16–18] showed that the pancreas produces a number of enzymes that were activated by bile, which entered the digestive system together with enzymes.

Significant data were obtained [19–20] and they indicated that the most dangerous are the proteolytic enzymes produced by a body in active form. If the process of their production and excretion into other organs of the digestive system is disturbed, enzymes are activated directly in the pancreas, which can lead to the development of pancreatitis and related complications in the digestive system. In our studies, a positive relationship was obtained between total bilirubin and protease (r=0.51) with adding sunflower oil, and a negative relationship was found between LDH and protease (r=−0.75) with adding soybean oil 30 minutes before feeding.

Liver enzymes are of great importance for the course of various biochemical processes in the body. In the experiment, the activity of liver enzymes normally used for differential diagnosis was studied. Indicator liver enzymes showed a high relationship between AST and protease (r=0.54) with adding palm oil, and between amylase and ALT (r=0.62) with adding linseed oil an hour after feeding. Since AST and ALT are the most informative analyzes for liver diseases, it is possible to predict the effectiveness of adding fats into the body of calves by determining the level of indicator enzymes and correlation [21–22].

Determining the activity of enzymes and their content in the body is one of the main diagnostic methods for various diseases [23]. Based on the created mathematical models and biochemical blood parameters, it is possible to calculate the effect of the fat component on the animal’s body during its growth and development.

The data obtained reveal that the correlation of amylase, lipase, and protease with the main biochemical parameters, may indicate the development of liver pathologies, inflammatory and necrotic processes in tissue cells, diseases of the digestive, cardiovascular system [24–26]. The results of such studies make it possible to determine what processes occur in the body of the animal.

5. Conclusion

According to the results of the studies, a high correlation between the digestive enzymes of the pancreas and biochemical blood parameters can be characterized taking into account the time factor and the fat component, which greatly affects the productivity of livestock farms. The dependent
relationship in the experimental groups was as follows: sunflower little (+) uric acid and lipase \((r=0.57)\), total bilirubin and protease \((r=0.51)\), glucose and lipase \((r=0.68)\), protease and total protein \((r=0.59)\), protease and albumin \((r=0.51)\), protease and total bilirubin \((r=0.63)\), protease and phosphorus \((r=-0.55)\), respectively. (–) AST amylase \((r=-0.54)\), ALT lipase \((r=-0.52)\), amylase–total bilirubin \((r=-0.68)\), amylase and iron \((r=-0.62)\). Palm oil (+) total protein and lipase \((r=0.58)\), glucose and protease \((r=0.51)\), AST protease \((r=0.54)\), iron amylase \((r=0.57)\), calcium and lipase \((r=0.63)\), negative gamma–GT and lipase \((r=-0.53)\). Soybean oil (+) protease and creatinine \((r=0.50)\), (–) urea lipase \((r=-0.52)\), LDH protease \((r=-0.75)\), iron and protease \((r=-0.58)\), amylase–iron \((r=-0.65)\) amylase–g–GT \((r=-0.64)\), amylase–ALT \((r=0.62)\), ALT protease \((r=0.56)\), ALT lipase \((r=0.52)\), cholesterol amylase \((r=0.55)\), phosphorus lipase \((r=0.523)\), (–) gamma–GT protease \((r=-0.54)\), glucose amylase \((r=-0.63)\), glucose protease \((r=-0.52)\). Thus, a pronounced positive relationship was observed over a period of time in sunflower (30–60 minutes), palm (60–90 minutes) and linseed (0–30 minutes) oils. Negative effects can be seen when using a diet supplemented with soybean oil. The developed mathematical models make it possible to predict the secretory function of the pancreas and the body’s response to various types of fats, with a database of biochemical parameters and the enzymatic activity of the pancreas.

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Ethical standards: All applicable international, national, and institutional guidelines for animal care and use have been followed.

References
[1] Lage R, Diéguez C, Vidal-Puig A and López M 2008 AMPK: a metabolic gauge regulating whole-body energy homeostasis Trends in Molec. Med. 14(12) 539–49
[2] Wendlinger C, Hammann S and Vetter W 2014 Various concentrations of erucic acid in mustard oil and mustard Food Chem. 153 393–7
[3] Lebedev S, Gavrich I, Gubajdullina I and Kvan O 2019 The effect of nanoparticles on mineral me-tabolism in the body of laboratory animals 19th Int. Multidisciplinary Sci. GeoConf. SGEM 1(2) 971–8
[4] Alvarenga R R, Zaneronimo M G and Pereira L J 2011 Lipoprotein metabolism in poultry World’s Poultry Sci. J. 67 431–40
[5] Oliveira J I, Uni Z and Ferket P R 2008 Important metabolic pathways in poultry embryos prior to-hatch World’s Poultry Sci. J. 64 488–49
[6] Zubitsov V A and Minevich I E 2015 The strategy of the technology development in forage production on the flax seeds and its products processing use J. of VNIIMZH 4(20) 72–9
[7] Donskova L A, Belyaev N M and Leiberova N V 2018 Fatty-Acid Composition of Lipids as Functional Purpose Indicator of Poultry Meat Products from: Theoretical and Practical Aspects Food Chem. and Hygiene 3(1) 4–10
[8] Lebedev S V, Gavrich I A, Shejda E V and Miroshnikov I S 2019 Effect of various fats on digestibility of nutrients in diet of calves IOP Conf. Ser. Earth and Environmental Sci. 341 012066
[9] Crescenzo R, Bianco F, Coppola P, Mazzoli A, Tussellino M and Carotenuto R 2014 Fructose supplementation worsens the deleterious effects of short-term high fat feeding on hepatic steatosis and lipid metabolism in adult rat Exp. Physiol. 99 1203–13
[10] Zhang G and Li C 2009 AMPK: an emerging drug target for diabetes and the metabolic syndrome Cell Metabolism 9(5) 407–16
[11] Nutrient Requirements of Beef Cattle: Seventh Revised Edition: Update 2000 Subcommittee on Beef
Cattle Nutrition, Committee on Animal Nutrition, National Research Council This PDF is available from the National Academies Press Retrieved from: http://www.nap.edu/catalog/9791.html

[12] Mao T, Yang L and Liu Y 2011 The mechanism of cell endoplasmic reticulum stress and glucose and lipid metabolism disorder Chinese J. of Cell Biol. 33(7) 727–37

[13] Lebedev S, Sheida E, Vertiprakhov V, Gavrish I, Kvan O and Gubaidullina I Z 2019 A study of the exocrinous function of the cattle pancreas after the introduction of feed with a various protein source in rations Biosci. Res. 16(3) 2553–62

[14] Walther T C and Farese R V Jr 2012 Lipid droplets and cellular lipid metabolism Annu. Rev. Biochem. 81 687–714

[15] Nakamura M, Nomura S and Yamakawa T 2018 Endogenous calcitonin regulates lipid and glucose metabolism in diet-induced obesity mice Sci. Reports 8 17001

[16] Li S and Hocking S L 2015 Subcutaneous fat transplantation alleviates diet-induced glucose intolerance and inflammation in mice Diabetol. 58 1587–600

[17] Zhou J, Liu H and Zhou S 2016 Adaptor protein APPL1 interacts with EGFR to orchestrate EGF-stimulated signaling Sci. Reports 61 1504–12

[18] Xie T, Li J and Mao T 2017 Decoction Ameliorates High-Fat-Induced Nonalcoholic Steatohepatitis in Rats by Regulating JNK1 Signaling Pathway Evidence-Based Complementary and Altern. Med. 14 4603701

[19] Gong Y Z, Sun S W and Liao D F 2017 Interaction and regulation of cell inflammation and lipid metabolism Chinese J. of Arterioscler 25(6) 623–9

[20] Pang J, Xi C, Huang X, Cui J, Gong H and Zhang T 2016 Effects of Excess Energy Intake on Glucose and Lipid Metabolism in C57BL/6 Mice Dairy Sci. 11(1) e0146675

[21] Dezhatkina S V, Lubin N A, Dosorov A V and Dezhatkin M E 2016 The use of soy okara in feeding of pigs Res. J. of Pharmac. cal Biolog. and Chem. Sci. 7(5) 2573–7

[22] Shaodong C, Haihong Z, Manting L, Guohui L, Zhengxiao Z and Ym Z 2013 Research of influence and mechanism of combining exercise with diet control on a model of lipid metabolism rat induced by high fat diet Lipids in Health and Disease 12(1) 21–3

[23] Yang HJ, Yim N and Lee KJ 2016 Simultaneous determination of nine bioactive compounds in Yijin-tang via high-performance liquid chromatography and liquid chromatography electrospray ionization-mass spectrometry Integrative Med. Res. 5(2) 140–50

[24] Fellmann L, Nascimento A R, Tibiriça E and Bousquet P 2013 Murine models for pharmacological studies of the metabolic syndrome Pharmacol. Ther. 137 331–40

[25] Debosch B J, Chen Z J, Finck B N, Chi M and Moley K H 2013 Glucose transporter-8 (GLUT8) mediates glucose intolerance and dyslipidemia in high-fructose diet-fet male mice Mol. Endo-crinoil 27 1887–96

[26] Kairenius P and Leskinen H 2018 Effect of dietary fish oil supplements alone or in combination with sunflower and linseed oil on ruminal lipid metabolism and bacterial populations in lactating cows Dairy Sci. 101(4) 3021–35

[27] Jing X, Jian-Bo Wan and Chengwei He 2014 Concise Review: Regulation of Stem Cell Proliferation and Differentiation by Essential Fatty Acids and Their Metabolites Stem. Cells 32(5) 1092–8