Biotechnological aspects of the use of vegetable oils in the production of meat products

E V Sheida, S V Lebedev, I Z Gubaidullina, V V Grechkina and V A Ryazanov
Federal Scientific Center for Biological Systems and Agrotechnologies of the Russian Academy of Sciences, Orenburg, Russia

E-mail: elena-shejda@mail.ru

Abstract. Biotechnological processes are becoming an important component of the world's economy, including agro-industrial production. They are based on the use of biological objects such as plants, animals and microorganisms; biological processes, the effective management of which is the essence of biotechnology in the traditional and modern sense. Biotechnology provides groundbreaking approaches to improving animal health and livestock productivity. This improvement is possible by improving the use of high quality feed, as well as by increasing the efficiency of their use in the body. This problem can be solved by including vegetable oils in full-component diets, which are concentrated sources of energy for the body as a whole and give the feed certain taste and structure. The paper shows the positive effect of the additional introduction of vegetable oils (soybean and linseed ones) on increasing the productive qualities of beef cattle.

1. Introduction
Increased demand for high-quality beef requires an intensive concentrated-feed cattle feeding system. From a physiological point of view, this type of feeding for polygastric animals is unconventional. It results in a large amount of lactate and short chain fatty acids, which leads to a decrease in pH in the rumen and disruption of fermentation processes, which in turn reduces the efficiency of meat production [1]. Vegetable and animal fats are an alternative source of energy. Nevertheless, there is evidence of a negative effect of oil additives in feed on scar microbiome, fermentation, dry matter digestibility and functional activity of the digestive glands [2, 3]. Impairment of the pancreas leads to a decrease in weight gain and, as a result, to a decrease in metabolic processes in animals’ bodies and their productivity. Therefore, it is necessary to concern the type of oil additives suitable for the gastrointestinal tract normal functioning. Linseed and soybean oils are considered the most preferable for feeding livestock and poultry and their role in digestion should be comprehensively evaluated [4].

2. Problem statement
To study the effect of soy and linseed oils on the activity of pancreatic enzymes.

3. Materials and research methods
3.1. Object of study
The object of study is the calves of the Kazakh white-headed breed aged 9 months with an average weight of 215–220 kg.
Animal care and experimental studies have been carried out in accordance with the instructions and recommendations of Russian Regulations, 1987 (Order No. 755 on August 12th, 1977 the USSR Ministry of Health) and “The Guide for Care and Use of Laboratory Animals (National Academy Press Washington, D.C. 1996)”.

3.2. Research Design
Animals were kept in separate metabolic cages (1.5×2.2 m) to collect urine, feces and pancreatic juice, in a room with optimal temperature and humidity for this type of animals with free access to water. During the experimental period, the ambient temperature was maintained between 23 and 25°C. The control calves during the experiment received a diet (T1), including mixed grass (2 kg), a mixture of concentrates (1.5), corn silage (5 kg), wheat straw (1 kg), molasses (0.1 kg), common salt (0.04 kg), vitamin and mineral premix (0.06 kg, elements per 1 kg of concentrates were as follows: Mn, 48 mg; Zn, 36 mg; Fe, 60 mg; Cu, 10 mg; Se, 0.24 mg; Co, 0.12 mg; vitamin content per kg of concentrate was as follows: vitamin A (VA), 2640 M; vitamin D (VD), 302 IU; Vitamin E (VE) 17 mg. Soybean oil was additionally added to the diet of group T2, linseed oil in the T3 group at the rate of 3 % of the dry matter of the diet by replacing the concentrate portion with the unrefined vegetable oils under study.

3.3. Pancreatic secretion
The exocrine pancreatic function was studied on 4 fistulated calves [5] at the age of 9 months with an average weight of 210-220 kg. The experiment was carried out in triplicates using the Latin 4x4 square in the laboratory of biological tests and examinations of the Federal Scientific Center for Biological Systems of the Russian Academy of Sciences. Juice was collected for 8 hours at intervals of 60 minutes. The studies were carried out in the laboratory “Agroecology of technogenic nanomaterials” and the Testing Center (Federal Scientific Center “Biological Systems and Agrotechnologies RAS”, accreditation certificate is RA. RU.21PF59 of December 2nd, 2015).

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

3.4. Statistical processing
Statistical analysis was performed using ANOVA techniques (Statistica 10.0 software package, “StatSoftInc.”, USA) and MicrosoftExcel. The significance of differences in the compared indicators was determined by Student’s t-test. The level of significant difference was set at P<0.05.

4. Research results
Over the reference period (8 hours) relative to the control diet, pancreatic juice secretion increased by 11.1 % with the incorporation of soybean oil and by 5.9 % with the inclusion of linseed oil (Fig. 1).

Changes in the qualitative characteristics of pancreatic secretion are presented in Table 1.

When soybean oil was included in the diet, lipase levels increased by 82.3 %, with linseed oil incorporation it increased by 60.5 % and remained active throughout the entire study period, with maximum values for 3 hours after feeding.

Amylolytic activity with soybean oil decreased by 41.1 % and with linseed oil by 1.8 times relative to the control group and maintaining the dynamics within 8 hours of the experimental study. Having incorporated linseed oil into the diet the activity of intestinal proteases was reduced only 60 minutes after feeding. Afterwards, this activity increased by 37.6 % (p≤0.05) in contrast to control. The level of protein changed diametrically to proteolytic enzymes. In case of soybean oil it increased almost by 2 times, and when giving food with linseed oil, on the contrary, it decreased by more than 2 times.
Figure 1. Amount of pancreatic juice secreted by pancreas when oils are incorporated in the diet, ml

Table 1. Dynamics of the pancreatic enzyme activity in calves treated with soybean and linseed oil
(n=5, M±m)

| Experience periods | Lipase         | Amylase mg/ml/min | Protease mg/ml/min | Total protein g/l | Phosphorus mmol/l | Calcium mmol/l | a-Amylase u/l |
|--------------------|----------------|-------------------|-------------------|------------------|------------------|----------------|---------------|
| 0-60 (before feeding) | 134±52.2 | 4800±669 | 140±19.4 | 0.42±0.05 | 0.14±0.01 | 2.34±0.03 | 416±10.5 |
| 60-120 (after feeding) | 82.2±3.45 | 2600±220 | 283±41.6 | 0.27±0.02 | 0.15±0.02 | 2.44±0.04 | 413±1.50 |
| 120-180 | 157±7.15 | 4400±800 | 183±16.6 | 0.73±0.07 | 0.07±0.07 | 2.28±0.02 | 463±1.00 |
| 180-240 | 184±33.2 | 5700±900 | 137±29.7 | 0.51±0.15 | 0.14±0.02 | 2.19±0.04 | 494±8.00 |
| 240-300 | 53±1.125 | 5700±900 | 91.7±33.3 | 0.41±0.07 | 0.16±0.03 | 2.31±0.14 | 383±4.50 |
| 300-360 | 24.9±1.95 | 5200±200 | 58.3±8.33 | 0.38±0.02 | 0.15±0.01 | 2.38±0.04 | 366±1.50 |
| 360-420 | 62.8±1.40 | 8000±600 | 87.5±4.17 | 0.34±0.12 | 0.14±0.01 | 2.35±0.05 | 418±6.00 |
| 420-480 | 24.4±1.12 | 4700±700 | 83.3±5.67 | 0.57±0.26 | 0.15±0.01 | 2.32±0.08 | 377±2.00 |
| 0-480 | 90.9±18.2 | 5137.5±450 | 133.5±24 | 0.46±0.12 | 0.14±0.02 | 2.33±0.12 | 416±3.48 |

I group

| Experience periods | Lipase         | Amylase mg/ml/min | Protease mg/ml/min | Total protein g/l | Phosphorus mmol/l | Calcium mmol/l | a-Amylase u/l |
|--------------------|----------------|-------------------|-------------------|------------------|------------------|----------------|---------------|
| 0-60 (before feeding) | 357±92.4* | 3078±267 | 117±9.22 | 0.85±0.08 | 0.16±0.05 | 1.76±0.12 | 2410±461* |
| 60-120 (after feeding) | 662±274 | 4000±100* | 76.3±34.7 | 0.82±0.22 | 0.16±0.01 | 2.09±0.4* | 2765±1041 |
| 120-180 | 703±239 | 3575±625 | 121±1.00 | 0.60±0.04* | 0.14±0.01 | 2.04±0.5* | 3055±1033 |
| 180-240 | 122±9.8* | 2200±400 | 119±6.00 | 1.10±0.02* | 0.16±0.01 | 1.57±0.5 | 1167±692* |
| 240-300 | 128±4.4* | 3500±100* | 116±16.7 | 1.09±0.3 | 0.16±0.01 | 1.33±0.5 | 137±709 |
| 300-360 | 187±9.6* | 2800±600 | 122±10.0 | 0.60±0.05 | 0.14±0.01 | 1.73±0.5 | 1453±634* |
| 360-420 | 202±76.0* | 1650±115 | 116±2.0** | 1.04±0.04* | 0.16±0.1 | 1.23±0.3 | 1120±422** |
| 420-480 | 513±126 | 3025±360 | 115±28.6 | 0.87±0.03 | 0.16±0.01 | 1.25±0.4 | 2197±896 |

II group

| Experience periods | Lipase         | Amylase mg/ml/min | Protease mg/ml/min | Total protein g/l | Phosphorus mmol/l | Calcium mmol/l | a-Amylase u/l |
|--------------------|----------------|-------------------|-------------------|------------------|------------------|----------------|---------------|
| 0-60 (before feeding) | 224±53.3 | 2587±416* | 186±23.7* | 0.18±0.05 | 0.11±0.01 | 2.41±0.6* | 1666±220* |
| 60-120 (after feeding) | 481±16.2** | 1800±200 | 225±50.0 | 0.13±0.15 | 0.11±0.04 | 2.53±0.18* | 1846±667 |
| 120-180 | 404±14.0 | 2000±200 | 287±70.8 | 0.16±0.67 | 0.12±0.05 | 2.60±0.51 | 1894±965 |
| 180-240 | 286±99.8 | 3100±700 | 170±20.8 | 0.01±0.006 | 0.14±0.05 | 2.55±0.84* | 1879±999 |
| 240-300 | 65.5±25.3 | 4600±400 | 229±45.5 | 0.39±0.08* | 0.12±0.01 | 2.38±0.98 | 1979±975 |
| 300-360 | 97.9±7.9 | 1400±800 | 154±70.8 | 0.31±0.02* | 0.12±0.01 | 2.16±1.02 | 1886±857 |
| 360-420 | 113±66.4 | 2800±600 | 204±12.5* | 0.36±0.06* | 0.12±0.01 | 2.20±1.03 | 1127±651* |
| 420-480 | 168.1±59.2 | 4700±300 | 250±41.7 | 0.04±0.01 | 0.12±0.01 | 2.31±0.94 | 1860±56* |
| 0-480 | 230±26.4 | 2874±700 | 214±36.1 | 0.21±0.03 | 0.12±0.01 | 2.4±0.78 | 1767±632 |

Saturation with macroelements in pancreatic juice was accompanied by an increase in phosphorus by 70%, against the background of a 27% decrease in Ca when soybean oil was included in the diet. The level of α-amylase activity relative to the control was higher throughout the entire experimental study.
Thus, the inclusion of vegetable oils in the diet of calves was accompanied by an increase in the activity of lipase and protease against the background of a decrease in amylase activity with an increase in the prosthetic-amylase and lipase-amylase ratios to 1:13 and 1:12, respectively (table 2).

Table 2. Ratio of main proteolytic enzymes in pancreatic juice of calves

| Diets          | Protease-amylase | Lipase-Amylase | Lipase-protease |
|----------------|------------------|----------------|-----------------|
| Control        | 1:38             | 1:56           | 1:1.4           |
| Soybean oil    | 1:26             | 1:5.8          | 4:1             |
| Linseed oil    | 1:13             | 1:12           | 1:1.1           |

The activity level of pancreatic enzymes in the duo denal chyme is characterized by a decrease in amylase activity of soybean oil by more than 2.7 times and of linseed oil by more than 1.6 times (Fig. 2).

The activity of lipase in the content of calves’ duodenum while incorporating oils was 15.4 % higher than in the control diet with the introduction of soybean oil and 18.5 % higher with the introduction of linseed oil. When the control diet was replaced with a soybean oil diet the activity of intestinal proteases increased by 28 %. While being replaced with the diet containing linseed oil, on the contrary, it decreased by 27.8 %.

**Figure 2.** Activity of pancreatic enzymes in content of calves’ duodenum when vegetable oils were included in the diet (n=5, M±m): (a) amylase enzyme activity, mg/ml/min; (b) enzyme lipase activity, U/l; (c) intestinal proteases activity, mg/ml/min
5. Discussion
The oils considered in this study, specifically, soybean and linseed ones, are considered suitable oils for use as an energy source in cattle nutrition. These oils have different compositions of fatty acids: soybean oil contains linoleic acid, linseed oil contains linolenic acid. Being a good source of energy on the one hand, oils exert pressure on the digestive system, on the other hand, by changing the functional activity of individual digestive organs involved in the regulation of the normal supply of the digestive conveyor. The pancreas is the organ that changes its pancreatic exosecretion in response to the transformation of constituent components in diets [6].

Pancreatic exosecretion is characterized not only by a large number of enzymes secreted by the iron but also by the ability to regulate their amount and ratio in the secretion content. It is ensured by adaptive synthesizing of the corresponding enzymes by acinocytes, and depending on the type of food received, provided by the preferential transport of the corresponding enzymes in the secretion [7]. When oils are included in the diet, pancreatic secretion is able to secrete the necessary set of enzymes for the hydrolysis of lipids supplied with food. Pancreatic lipase breaks down up to 90% of food-taken lipids previously emulsified by bile [8–12]. Thus, an increase in pancreatic juice secretion was noted in response to the introduction of soybean and linseed oil into the diet of cattle, as well as an increase in the level of activity of the lipase enzyme and intestinal proteases against the background of a decrease in the amylolytic activity of pancreatic secretion [13, 14].

6. Conclusion
When soybean and linseed oils were introduced into the diet of cattle during the reference period, 417 ml of pancreatic juice was egested with incorporating soybean oil into the diet, and 390 ml of pancreatic juice was egested with incorporating linseed oil. Soybean and linseed oils had a milder effect on the synthesis of enzymes and were characterized by an increase in the activity of lipase and intestinal proteases, maintenance of the lipase-protease ratio (4:1), and a 2-fold increase in prosthetic-amylase ratio. When linseed oil was introduced into the diet, the activity of all enzymes decreased with a maximum of 70% lipase synthesis (lipase-protease ratio was equal to 1:1.1, prosthetic-amylase and lipase-amylase increased to 1:13 and 1:12, respectively). Pancreatic juice has varied ambiguously in response to the introduction of oils. Thus, an increase in the level of total protein and P by 12.5% and 70% respectively was observed against the background of a decrease in Ca by 27% with the incorporation of soybean oil in the diet. When soybean oil was replaced with linseed oil, the Ca level increased by 3%, while the concentration of total protein and P, on the contrary, decreased by 54.3% and 14.3% respectively. The activity of lipase and amylase in the chyme decreased and protease increased under the conditions of incorporating soybean oil in the diet.

Acknowledgments
The studies were carried out in accordance with the research plan for 2019–2020 of the Federal Research Center for Biological Systems and Agrotechnology’s of the Russian Academy of Sciences (№ 0761-2019-0005).

Conflict of Interest: The authors declare that they have no conflict of interest.

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

References
[1] Cheng K J, McAllister T A, Popp J D et al 1998 A review of bloat in feedlot cattle J. Anim. Sci. 76 299–308 Retrieved from: https://doi.org/10.2527/1998.761299x
[2] Abubakr A R, Alimon A R, Yaakub H et al 2013 Digestibility, rumen protozoa, and ruminal fermentation in goats receiving dietary palm oil by-products J. Saudi Soc. Agric. Sci. 12 147–54 Retrieved from: https://doi.org/10.1016/j.jssas.2012.11.002
[3] Abubakr A R, Alimon A R, Yaakub H et al 2014 Effect of feeding palm oil by-products based diets on total bacteria, cellulolytic bacteria and methanogenicarchaea in the rumen of goats Plos. One 9 e95713 Retrieved from: https://doi.org/10.1371/journal.pone.0095713

[4] Lebedev S V, Gavrish I A, Shejda E V et al 2019 Effect of various fats on digestibility of nutrients in diet of calves Conference on Innovations in Agricultural and Rural development IOP Conf. Ser. Earth and Environmental Sci. 341 012066. DOI: 10.1088/1755-1315/341/1/012066

[5] Sheida E, Sipailova O, Kvan O, et al 2014 Functional properties of antimicrobial peptides extracted from hens' platelets Life Science Journal 11(9) 25, 180-184

[6] Korotko G F 2007 The role of pancreatic exocrine secretion in the digestive conveyor Bull. of surgical gastroenterol. 3 35–41

[7] Lebedev S, Sheida E, Vertiprakhov V et al 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

[8] Lebedev S, Gavrish I, Rusakova E et al 2018 Influence of various chromium compounds on physiological, morpho-biochemical parameters, and digestive enzymes activity in Wistar rats Trace elements and electrolytes 35(4) 242–5 Retrieved from: https://doi.org/10.5414/TEX0155419

[9] Carriere F, Barrowman J A, Verger R and Laugier R 1993 Secretion and contribution to lipolysis of gastric and pancreatic lipases during a test meal in humans Gastroeneterol. 105 876–88

[10] Carriere F, Renou C, Ransac S, Lopez V et al 2001 Inhibition of gastrointestinal lipolysis by Orlistat during digestion of test meals in healthy volunteers Am. J. Physiol. Gastrointest. Liver Physiol. 281 16–28

[11] Plichiewicz A, O’Donovan D, Feinle C, Lei Y et al 2003 Effect of Lipase Inhibition on Gastric Emptying of, and the Glycemic and Incretin Responses to, an Oil, Aqueous Drink in Type 2 Diabetes Mellitus J. Clin. Endocrinol. Metabol. 88(8) 3829–34

[12] Roussel A, Canaan S, Egloff M P, Rivie`re M et al 1999 Crystal Structure of Human Gastric Lipase and Model of Lysosomal Acid Lipase, Two Lipolytic Enzymes of Medical Interest J. of Biol. Chem. 274(24) 16995–7002

[13] Duskaev G, Karimov I, Levakhin G et al 2019 Ecology of ruminal microorganisms under the influence of quercus cortex extract International Journal of GEOMATE 16(55) 59-66

[14] Duskaev G K, Rakhmatullin S G, Kazachkova N M, Sheida Y V et al 2018 Effect of the combined action of Quercus cortex extract and probiotic substances on the immunity and productivity of broiler chickens Veterinary World 11(10) 1416-1422