Lipid spectrum of blood when vegetable fats are introduced into the diet of calves

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Abstract. The study of metabolic effects of lipophilic vegetable products: sunflower (I group, n=3), palm (II group, n=3) and linseed oil (III group, n=3) were carried out on calves at the age of 9 months (n=3) with a body weight of 215–220 kg. The introduction of sunflower and palm oil was expressed by the increase in high density lipoproteins H\textsubscript{S}-HDL and OHs. According to H\textsubscript{S}-HDL/H\textsubscript{S}-LDL accumulation ratio, the test fats were arranged in the groups as follows: II (1.82)>III (1.78)>I (1.74)>control (0.77) conv. units. The effect of vegetable fats on the blood serum of experimental animals according to OHs/Hs-HDL index was as follows: III (0.34)<I (0.36)<I (0.38)<control (0.46). Thus, the introduction of oils reduces LDL and the atherogenic index in blood serum with a significant increase in HDL. This can serve the basis for the use of vegetable oils as promising natural hepatoprotectors in feeding animals, and at the stage of growing it will help to monitor the level of lipids in the body of calves and to calculate them in diet formulation.

1. Introduction
The growth, development, productivity and metabolism of animals depend on many factors, one of the key being proper and adequate feeding. Due to the development of livestock production, an important task is to obtain high-quality products and this is impossible without a full feed base. It is the balanced feed that makes it possible to obtain high level of productivity, to reduce feed consumption and to contribute to the body in general [1–3].

Currently, for farm animals and birds, liquid forms of fats are more commonly used in diet formulation. But recent research showed that the use of dry forms of vegetable and animal fats in livestock breeding is the most technologically beneficial. The production of dry fats does not require the use of additional equipment and financial investments [4].

One important indicator in animal feeding is lipidic nutritional content of feed. When growing animals and poultry, fat additives are one of the main diet components. A certain set with the minimum content of essential linoleic acid is used in the production of complete mixed fodders [5–7].

A number of foreign and Russian researchers proved that metabolism normalization in the animal body is related to the regulation of the nervous system [8], the level of hormones [9–10] and enzymes [11], as well as to the age and breed of animals [12]. It is important to determine the concentration of hormones and enzymes in the living organism in order to treat and prevent various diseases [13–15].

Therefore, the analysis of the lipid blood spectrum is important in the normal feeding of animals and in the additional introduction of vegetable oils into the body of calves.
2. Materials and methods

2.1. Animals and feed

The study was carried out in the Laboratory of Biological Experiments and Expertise of the Federal Scientific Center of Biological Systems and Agricultural Technology of the Russian Academy of Sciences on calves of Kazakh Whiteheaded calves ($n=3$) with the average weight of 215–220 kg, at the age of 9 months.

All animal services and experimental studies were carried out in accordance with instructions and recommendations of the Russian Regulations, 1987 (Order No. 755 of 12.08.1977 the USSR Ministry of Health) and The Guide for Care and Use of Laboratory Animals (National Academy Press Washington, D.C. 1996). The calves were kept in a specialized cage with free access to water and food.

Feeding of animals was carried out through the basic diet compiled taking into account the NRC recommendations (www.nap.edu/catalog/9791.html) and included the following: mixed hay (2 kg), mixture of concentrates (1.5), maize silage (5 kg), wheat straw (1 kg), beet molasses (0.1 kg), cooking salt (0.04 kg), vitamin-mineral premix (0.06 kg, micronutrient content per 1 kg of concentrates: Mn – 48 mg; Zn – 36 mg; Fe – 60 mg; Cu – 10 mg; Se – 0.24 mg; Co – 0.12 mg; vitamin content per 1 kg of concentrates: vitamin A (VA) – 2640 M; vitamin D (VD) – 302 MU; vitamin E (VE) – 17 mg.

The control group calves received the basic diet throughout the experiment. The basic diet of the I experimental group included sunflower oil (extra virgin, grade A), II experimental group – palm oil (GOST 31647-2012), III experimental group – linseed oil (STO 40490379-001-2015, TR TS 024/2011). The vegetable oil was added in the amount of 3 % of the dry matter due to the cereal portion of the diet.

2.2. Observations, measured indicators

The blood for biochemical parameters was collected from the jugular vein into vacutainer vacuum tubes with coagulation activator (thrombin). The diagnostics was carried out in the Laboratory of Agroecology of Technogenic Nanomaterials and the Test Center of Biological Systems and Agricultural Technologies of the Russian Academy of Sciences, accreditation certificate RA.RU.21PF59 of 02.12.15). Total lipids (OL), total cholesterol (OHs), high density lipoprotein cholesterol (Hs-HDL), low density lipoprotein cholesterol (Hs-LDL), triacylglycerides (TAG) were analyzed using automatic CS-T240 analyzer (DIRUI IndustrialCo., China) with the commercial veterinary set (ZAO DIAKON-DS, Russia). Cholesterol coefficient of atherogenicity (CAt) was calculated by the formula:

\[
CAt = \frac{OHs-Hs-HDL}{Hs-HDL}
\]

The content of low and very low density lipoproteins was calculated by the formula:

\[
Hs-VLDLP = \frac{Otg}{2.2}
\]

\[
Hs-LDL = OHs-Hs-HDL-Hs-VLDL
\]

2.3. Statistical processing

Statistical analysis was performed using ANOVA (Statistica 10.0, StatSoftInc, USA) and Microsoft Excel. The statistical significance of the difference between comparative values was determined by the Student criterion. The values at $p<0.05$ were considered reliable. The data are presented as the arithmetic mean ($M$) and the standard error of the arithmetic mean ($m$).

3. Results

One important indicator of lipid metabolism is the amount of cholesterol in the blood serum of animals. The main transport form of cholesterol in the blood is low density lipoproteins (LDL) delivering cholesterol to peripheral cells (Table 1).
Table 1. Blood composition of experimental calves

| Indicator               | Calf groups       |
|-------------------------|-------------------|
|                         | control          | I-experimental | II-experimental | III-experimental |
| Total lipids (OL), g/l  | 1.08 ± 1.16*     | 1.96 ± 0.13    | 2.09 ± 0.11     | 1.18 ± 1.06*     |
| Triacylglycerides (TAG), mmol/L | 0.26 ± 0.03* | 0.43 ± 0.06    | 0.48 ± 0.05     | 0.22 ± 0.03*     |
| TAG/OL, conv. units     | 0.24 ± 0.04       | 0.22 ± 0.03    | 0.23 ± 0.05     | 0.21 ± 0.04      |
| Total cholesterol (OHs), mmol/l | 2.00 ± 0.14* | 2.86 ± 0.05    | 2.91 ± 0.17     | 2.07 ± 0.14*     |
| OHs /OL, conv. units    | 1.39 ± 0.10*      | 1.49 ± 0.11    | 1.59 ± 0.15     | 1.45 ± 0.10*     |

*Differences with control are reliable at $p<0.05$

The concentration of total lipids, triacylglycerides, total cholesterol and cholesterol in high and low density lipoproteins varies within 1.96–2.09 g/l, 0.43–0.48 mmol/L, 2.86–2.91 mol/L, 1.98–2.13 and 1.14–1.17 mmol/L, respectively.

Against the background of a slight change in the concentration of OHs from 2.86 to 2.91 mmol/L in the blood of calves of the I and II experimental groups, a constant level of HS-HDL, HS-LDL and their ratio was observed (Table 2).

At the same time the OHs blood level in the animals of the III experimental group was reduced against the background of the increase of HS-LDL content by 1.43–1.46 times ($p<0.05$) and the reduction of HS-HDL by 1.55–1.66 times ($p<0.05$). The increase of this indicator demonstrates more intensive fat deposition.

Table 2. Transport forms of cholesterol

| Indicator               | Calf groups       |
|-------------------------|-------------------|
|                         | control          | I-experimental | II-experimental | III-experimental |
| HS-HDL, mmol/l          | 1.28 ± 0.09*     | 2.06 ± 0.07    | 2.13 ± 0.11*    | 1.98 ± 0.06      |
| HS-LDL, mmol/l          | 1.67 ± 0.08*     | 1.14 ± 0.14    | 1.17 ± 0.09     | 1.12 ± 0.04*     |
| HS-HDL / HS-LDL, conv. units | 0.77 ± 0.08* | 1.74 ± 0.28    | 1.82 ± 0.15 *   | 1.78 ± 0.02      |

*Differences with control are reliable at $p<0.05$

In complex with cholesterol, phospholipids and apoproteins, triglycerides are included in the composition of very low density lipoproteins (VLDL). The definition of the atherogenic index serves a more reliable evidence of lipid metabolism than the increase or decrease in the total triglyceride content (Figure 1).

Figure 1. Coefficient of atherogenicity of experimental calves, $p<0.05$
Under the influence of vegetable fats, the experimental calves experienced the normalization of lipid composition of blood. LDL decreased in the experimental groups as follows: I (by 31.74 %), II (by 29.94 %), III (by 32.93 %), resulting in the following coefficient of atherogenicity: I (0.36), II (0.38), III (0.34). The CAt ratio in the calves of the III experimental group was 30.61 % lower than that of the control group.

Thus, by the degree of HDL/LDL accumulation, the studied fats can be distributed in the following order: II (1.82)>III (1.78)>I (1.74)>control (0.77) conv.units, and by the OHs/HDL atherogenic index in blood serum: III (0.34)<I (0.36)<II (0.38)<control (0.46).

4. Discussion

Blood indices indicate the work of the whole organism, they can characterize the level of adaptation of animals to various stress factors, including to the specific conditions of animal housing and feeding [16].

The participation of vegetable oils in lipid exchange was expressed by the LDL decrease in the experimental groups of calves alongside with the atherogenic index: I (31.74 %), II (29.94 %), III (32.92 %), which may be caused by their participation in atherogenesis: ongoing circulation in the bloodstream, easy oxygen demand, binding to the proteoglycans of the arterial wall, good ability to penetrate the endothelial barrier. LDL represent spherical particles, heterogeneous in lipid and protein composition, density and diameter, sensitivity to oxidation and non-contactable absorption by macrophages, high ability to penetrate into subendothelial space of the vascular wall [17].

The inclusion of sunflower fat was found to contribute to the decrease OHs and Hs-LDL levels in plasma and is consistent with the previous studies [18–19]. The results of the study [20] showed that the increase in the TAG level is less dangerous than hypercholesterolemia causing excessive accumulation of free circulating low-density lipid complexes in the blood.

The inclusion of palm fat into a diet was followed by the increase in triglycerides – by 55.17 % and cholesterol – by 69.99 % (р0.05), similar to earlier obtained data [21] where the inclusion of complex feeding on the basis of palm oil normalizes and stimulates the exchange of protein and lipid substances, including the concentration of the total protein, albumens and globulins, immunoglobulins, activity of transamination enzymes. At high HDL levels, the LDL content simultaneously increased thus affecting the atherogenicity index in the calves receiving palm fat [22].

Metabolic diseases in animals are associated with the change in lipid indicators such as LDL and high cholesterol levels. These disorders are accompanied by abnormal biochemical indicators, which are generally accompanied by elevated levels of total cholesterol and triglycerides. The atherogenic index more accurately reflects the favorable and unfavorable combination of lipoproteins in terms of the development of atherosclerosis [23–27].

On the one hand, the increase of HDL level is a prognostic factor of atherosclerosis, and the low level of HDL is related to the risk of atherosclerosis and mortality, on the other hand, the clinical studies showed that the increase of the HDL level in the I and II experimental groups did not reduce the risk of cardiovascular diseases. Therefore, HDL increase is only important with simultaneous LDL decrease, which is reflected in the atherogenic index [28]. The use of linseed oil contributed to the improvement of these conditions and the decrease of LDL and the atherogenic index in the blood serum [29].

The obtained data on lipid exchange are consistent with the results of the studies, which showed that the flax extract positively affects biochemical indices of human blood in case of hepatitis and increases the productivity of animals [30–32].

5. Conclusion

Thus, the administration of oils has a pronounced effect on lipid metabolism in the calves of the experimental groups, which is expressed in the decrease of LDL and the atherogenic index in blood serum, with a significant increase of HDL. By changing IA, it is possible to assume the development of pathological conditions that can lead to the disruption of lipid metabolism. In terms of Hs-HDL/Hs-
LDL accumulation ratio, the experimental groups of calves received a diet with soybean and palm fat: II (1.82) > III (1.78) conv.units. High effect of vegetable fats on the body of experimental animals regarding the atherogenic index OHs/HS-HDL in the blood serum was low in the calves of experimental groups receiving palm fat (II – 0.38, III – 0.34). This can serve the basis for the use of vegetable oils as promising natural hepatoprotectors in feeding animals, and at the stage of growing it will help to monitor the level of lipids in the body of calves and to calculate them in diet formulation.

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