

# USE OF MARE'S MILK IN THE TREATMENT OF NON-ALCOHOLIC STEATOHEPATITIS

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## Abstract

Non-alcoholic steatohepatitis (NASH) is an advanced form of non-alcoholic fatty liver disease (NAFLD) and is usually associated with overweight, obesity and metabolic syndrome. The major treatment offered for NAFLD is a healthy lifestyle, calorie restriction diet and regular physical activity. In this regard, mare's milk may be considered a pathogenetically substantiated, highly effective and affordable natural health product or dietary supplement for NASH patients. Mare's milk is known for its high value due to its similarity to human breast milk in chemical composition aspects. Through a review of the literature on mare's milk, this article analyzes the medical and dietary potentials of sublimated mare's milk for NASH patients. In this study, we used a specifically developed questionnaire, as well as clinical, biochemical and ultrasound methods.

**Keywords:** non-alcoholic steatohepatitis, non-alcoholic fatty liver diseases, mare's milk, sublimated mare's milk, medical-dietary properties

## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disorder worldwide. NAFLD may be considered as the hepatic component of metabolic syndrome, affecting approximately 20-33% of entire adult population [1,2]. Non-alcoholic steatohepatitis (NASH) is an advanced form of NAFLD and strongly associated with overweight, obesity and metabolic syndrome [3]. Unhealthy lifestyles, poor nutrition and lack of physical activity are the main risk factors for developing NAFLD [4].

The pathogenesis of NASH involves free fatty acids and triglycerides accumulation in the liver, as well as lipid peroxidation, which leads to the accumulation of toxic intermediates and then, developing into liver inflammation and fibrosis [3].

Underlying health conditions offered for NASH treatment or prevention are a healthy lifestyle, regular physical activity and a calorie restriction diet [3,4]. It is important that NASH patients follow a low-calorie diet with a reduced intake of saturated fatty acids (SFA), cholesterol, fructose and simple carbohydrates, as well as with higher level of polyunsaturated fatty acids (PUSFA), prebiotics and probiotics, and natural antioxidants as vitamins A, C, E and trace elements (Zn, Se, Mn, Cu, etc). In this regard, for NASH patients, mare's milk is a unique product, since it is a valuable element of nutrition therapy, and also has a high therapeutic potential for all NAFLD patients.

Mare's milk, also named Saumal, is used for centuries as a nutritional drink, due to its well-known health benefits [5]. In various countries of the world, mare's milk has been shown to be effective in treating digestive or liver diseases [6]. Mare's milk has high nutritional and medical properties. When used regularly, it helps alleviate or eliminate gastrointestinal symptoms. Also, mare's milk is useful for patients with chronic liver diseases, including hepatitis [7].

Freshly drawn mare's milk is a perishable and unstable product, which should be used within 3 hours after milking. In view of this, to date, no clinical trials describing the healing properties of mare's milk have been conducted. However, the development of innovative technology in creating sublimated pasteurized mare's milk with a long shelf life even when stored at room temperature, made it possible to conduct such researches.

Eurasia Invest Ltd LLP, an enterprise located in the Osakarovskiy district of the Karaganda Oblast', is a large-scale producer of Saumal, currently operating with the assistance of Kurgestüt Hoher Odenwald (Hans Zollmann), the German horse breeding farm. They produce Saumal using innovative sublimation dehydration technology in strict accordance with European quality standards [8].

## OBJECTIVES.

The purpose of this study was to provide a scientific basis for the substantiation and to study the effectiveness of mare's milk as a dietary supplement for NASH patients.

## MATERIAL AND METHODS

In order to achieve the above-mentioned objectives, the following study was carried out: 1) a literature review regarding the qualitative composition of mare's milk; 2) analysis of a survey of NASH patients taking Saumal using a specifically developed questionnaire; 3) processing of clinical, laboratory and ultrasound data collected from NASH patients while taking Saumal.

The medical-dietary properties of mare's milk was studied using the samples of Saumal produced by Eurasia Invest Ltd LLP (Osakarovskiy District, Karaganda Oblast', Kazakhstan). In relation to this milk, Expert Findings of independent laboratories in Russia (test laboratory center of the Head Center for Hygiene and Epidemiology of Russia, Moscow)

and China (SGS-CSTC Standards Technical Services Co., Ltd, Shanghai) have been issued. These findings reflect the quality of Saumal composition, the absence of harmful toxic agents and the similarity of its components with fresh mare's milk [8].

In order to determine the organoleptic properties, tolerance and therapeutic efficacy of Saumal, we conducted a survey of NASH patients taking mare's milk, according to a questionnaire specifically developed for this purpose. This survey involved 52 NASH patients consisted of 27 males and 25 females ranging in age between 26 and 60. According to Research Protocol, respondents took Saumal after dissolving 20 grams of the substance in 150-200 ml of warm water, 3 times a day for 2 months.

The survey and clinical trials involved outpatients from medical institutions in the cities of Nur-Sultan and Almaty, that were previously diagnosed with NASH. Patients were surveyed three times: 3-4 days after the start of Saumal taking, a month later, and at the end of the course.

Clinical, laboratory and ultrasound studies were conducted with the participation of 41 NASH patients consisted of 21 males and 20 females ranging in age between 36 and 60. The study was open, comparative, controlled, and randomized. Patients were randomly divided into two groups: 1) the main group (n=22) - NASH patients taking Saumal only; 2) the control group (n=19) - NASH patients taking ursodeoxycholic acid (Ursosan) as hepatoprotective monotherapy.

The main NASH syndromes, such as asthenic, pain and dyspeptic, were selected for the study of clinical symptomatology. In the study of laboratory parameters, transaminases (ALT, AST), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), bilirubin, cholesterol, low density lipoproteins (LDL), high density lipoproteins (HDL), triglycerides (TG) and glucose were involved. In addition, standard ultrasound parameters of the hepatobiliary system were studied - both before and after the end of Saumal course. For the sake of greater clarity, biochemical studies were carried out after 2 weeks after starting the course, after a month and at the end of therapy.

This study was carried out in the framework of the Scientific Grant (IRN AR051355855) of the Committee of Science of the Ministry of Education and Science of the Republic of Kazakhstan on the 'Life Science' priority. Before starting our research, we obtained patients' consent regarding the study and Findings of the local Research Ethics Committee. This research project is registered in the Clinical Trials.gov database.

### RESULTS AND DISCUSSION

Based on the results of a literature review on the quality composition of mare's milk, we received the following product characteristics. Mare's milk (Saumal) is a natural food product of a bluish-white color with a neutral reaction (pH 7.0—7.2), with a slightly astringent and sweet taste, and high medical-dietary properties. Regarding its protein composition, mare's milk is quite similar to that of human breast milk. Both they belong to so-called albumin milks, due to their high albumin content in relation to the casein [5].

The total amount of protein in mare's milk is 1.85-2.20%. With that, mare's milk casein is easily soluble in water, which points to its better digestibility, absorption and accessibility, compared with cow's milk [5,6]. Moreover, mare's milk proteins are well balanced in amino acid composition and do not cause an allergic reaction [9,10].

Saumal also contains lactoferrin, lysozyme and immunoglobulin. Lysozyme and lactoferrin play important roles in the human immune system defense, protecting the body from pathogens and killing bacteria in milk itself and then, in digestive system. Lysozyme constituted 5% of the

total protein, plays a particularly significant role in the bactericidal effect of mare's milk. In addition to its enzymatic action, lysozyme has anti-inflammatory, antiviral, immunostimulating, antitumor and anticancer properties [5,11,12].

Mare's milk also contains enzymes such as amylase, catalase, lipase, peroxidase, phosphatase, malate and lactate dehydrogenase, lactotransferrin, which contribute to the digestion process and support the body's defense system. Milk lactoferrin has antibacterial and antioxidant properties, as well as immunomodulatory and anti-inflammatory effects [5,11,12].

Immunoglobulins are protein compounds that play essential roles in immune defenses. In mare's milk, the predominant serum IgG isotypes are Ig A, Ig M, and Ig G, which neutralize viruses and toxins, prevent the attachment of bacteria to the surface of the epithelium, and activate leukocyte phagocytosis [11,12]. Mare's milk contains about 20% Ig, which is 1% more than in human breast milk [12].

Mare's milk fat has a higher biological value than that of cow's milk. Specifically, mare's milk fat is rich in polyunsaturated acids (PUSFA), which are mainly represented by essential fatty acids (linolenic and linoleic). Their specific gravity in the total fat molecule is 10-12%.

Carbohydrates in mare's milk are mainly represented by lactose, consisting mainly of  $\beta$ -lactose [5,13]. In contrast to  $\alpha$ -lactose,  $\beta$ -lactose absorption in the small intestine is slower; therefore, it enters the large intestine, where stimulates the growth of intestinal microflora, i.e. mainly gram-positive bacteria. Thus, due to the action of  $\beta$ -lactose, which is actually a bifidogenic and lactogenic prebiotic, mare's milk has a bifidogenic effect, normalizing intestinal microflora [5]. Mare's milk has a unique chemical composition close to that of human breast milk. Saumal is rich in vitamins A, B, D, E, as well as vital trace elements (Ca, K, Na, I, Co, Zn, Mn, Cu, Fe, Al...). Compared to other products of animal origin, vitamin C content in mare's milk is much richer. For example, 250 ml of mare's milk contains the recommended daily amount of vitamin C for adults [14]. *The content of vitamins E, B, B<sub>12</sub>, niacin* in mare's milk is also higher than in cow's milk. Due to the combination of these vitamins with ascorbic acid, Saumal has a positive impact on some chronic conditions [5].

After a survey of patients taking Saumal, we analyzed the data collected and obtained the following product characteristics and clinical results:

- 1) Saumal was described as having a pleasant flavour, with the slightly sweet taste of baby food and milk scent. The product is soluble in warm water easily and without curds, it quickly acquires a homogeneous texture of fresh milk,
- 2) Due to the high nutritional properties of mare's milk, patients reduced their daily calorie intake,
- 3) Decrease in pain and feeling of heaviness localized in the right hypochondrium,
- 4) Reducing dyspepsia syndrome - bitterness in the mouth, nausea, bloating,
- 5) Improving sleep quality (decreasing sleep latency, continuous consolidated sleep every night),
- 6) Feeling of lightness, more toned body and a well-being improvement,
- 7) Endurance and stamina increasing, reducing tiredness and fatigue,
- 8) Laxative effect when starting intake of the product, with a further normalization of bowel movement (after 3-5 days),
- 9) Weight loss and reduction in waist,
- 10) Some patients reported improving their skin's texture (softer skin), and reduction in existing skin rashes.

When conducting clinical, laboratory and ultrasound studies

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in NASH patients, all respondents reported improvement in their clinical symptoms, but the most significant improvement was found in the main group (Table.1). For example, after the 2-month course of Saumal was completed, the pain syndrome present in 45.5±2.1% of patients of the main group, manifested as sensation of heaviness and discomfort in the right hypochondrium, almost disappeared. In the control group, after the Saumal course was completed, this syndrome persisted in 10.5±1.8% of patients (p<0.01). After the course of taking Saumal, asthenic and dyspeptic syndromes significantly decreased (p<0.01). However, in the

control group, a decrease in these indices was insignificant, compared with those of the main group. Thus, asthenic syndrome decreased insignificantly (p>0.05), and decreasing of dyspeptic syndrome was statistically significant, but less than that in the main group (p<0.05). Hepatomegaly detected by ultrasound before Saumal course was observed in 90.9±2.4% of patients. After the Saumal course, its frequency decreased by 13.6%, amounting to 77.3±2.8 % A study in the control group showed that Ursosan did not affect hepatomegaly.

**Table 1.** Baseline and control clinical parameters

| No. | Clinical parameters/ syndromes | Saumal (n=22) |                     | Ursosan (n=19) |                     |
|-----|--------------------------------|---------------|---------------------|----------------|---------------------|
|     |                                | Baseline (%)  | After treatment (%) | Baseline (%)   | After treatment (%) |
| 1   | Pain                           | 45,5±2,1      | -                   | 42,1±2,0       | 10,5±1,8**          |
| 2   | Asthenic                       | 86,4±2,2      | 40,9±2,2**          | 89,5±2,1       | 73,7±2,3            |
| 3   | Dyspeptic                      | 77,3±2,3      | 36,4±2,3**          | 84,2±2,4       | 63,2±2,4*           |
| 4   | Hepatomegaly                   | 90,9±2,4      | 77,3±2,8            | 73,7±2,3       | 73,7±2,3            |

\* - significant difference in dynamics since the beginning of the course (p<0.05)  
 \*\* - significant difference in dynamics since the beginning of the course (p<0.05)

The laboratory study also revealed an improvement in biochemical parameters in the study groups, but more significant changes were found in the experimental group (Table.2). Thus, in the main group of patients (Saumal), in relation to the beginning of the course, the cytolysis indices significantly decreased: ALT (p<0.01) and AST (p<0.05). Compared with the start of Saumal course, the cytolysis indices in the control group (Ursosan) also decreased, but were statistically insignificant (p>0.05). In addition, GGT and ALP decreased in both groups, but they were statistically insignificant (p>0.05), except for changes in GGT in the control group (p<0.05). This fact is explained by the specific anti-cholestatic properties of UDCA (Ursosan).

Mare's milk stands out due to its positive impact on cholesterol metabolism. In this study, we also observed a decrease in cholesterol, LDL and TG (Table.2). In the control group of patients, these indices did not change, which was due to UDCA (Ursosan).

*Summarizing the above, we can conclude that the literature review [1-14] on Saumal rich biological composition (high albumin and PUFA, the presence of lysozyme, immunoglobulin and β-lactose, the optimal balance of antioxidants - lactoferrin, vitamins A, C, E and trace elements - Zn, Mn, Cu, etc.) points to its medical-dietary properties.*

A survey of patients taking Saumal showed that this product corresponds to full mare's milk in terms of its physical and

organoleptic properties. Saumal was well tolerated in all patients, with no serious side effects. Along with a reduction in pain, dyspepsia and asthenic syndromes, patients reported improvement in health, sleep and the skin's texture, reduction in existing skin rashes, and weight loss.

In addition, the results of clinical, laboratory and ultrasound studies showed a significant improvement in the clinical and biochemical syndromes of NASH while taking Saumal for 2 months.

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**Table 2.** Baseline and control biochemical parameters

| Biochemical parameters | Saumal (n=22) |               |              |                | Ursosan (n=19) |               |              |                |
|------------------------|---------------|---------------|--------------|----------------|----------------|---------------|--------------|----------------|
|                        | Baseline      | After 2 weeks | After months | After 2 months | Baseline       | After 2 weeks | After months | After 2 months |
| ALT                    | 46,97±2,9     | 38,3±6,1      | 33,3±4,2     | 30,5±2,5**     | 71,3±32,3      | 39,2±7,3      | 35,3±5,1     | 32,7±5,4       |
| AST                    | 68,9±7,3      | 57,1±0,2      | 49,1±7,7     | 43,1±4,6*      | 100,7±37,6     | 61,2±8,8      | 46,7±7,7     | 41,8±8,1       |
| Bilirubin total        | 13,8±1,9      | 12,3±2,0      | 12,9±2,4     | 11,8±2,2       | 13,8±2,3       | 14,6±2,1      | 11,4±1,8     | 12,5±2,2       |
| Bilirubin direct       | 5,6±0,8       | 5,5±0,8       | 6,6±1,1      | 6,0±0,7        | 5,1±1,1        | 4,1±1,0       | 3,3±0,6      | 3,8±0,9        |
| GGT                    | 66,3±9,8      | 50±6,4        | 47,1±7,4     | 43,6±5,8       | 97,5±29,5      | 83,3±23,8     | 76,5±19,8    | 74,1±17,5*     |
| ALP                    | 95,7±21       | 96,2±0,8      | 99,7±24,4    | 90,1±15,9      | 123,5±12,9     | 120,0±13,4    | 116,2±9,4    | 119,7±10,2     |
| Cholesterol            | 5,4±0,3       | 5,1±0,3       | 5,1±0,3      | 4,7±0,3        | 5,2±0,4        | 65,4±0,1      | 5,7±0,5      | 5,6±0,4        |
| HDL                    | 1,8±0,3       | 1,8±0,4       | 2,0±0,5      | 1,5±0,3        | 1,7±0,4        | 1,7±0,4       | 1,6±0,4      | 1,5±0,4        |
| LDL                    | 3,1±0,2       | 3,1±0,2       | 2,9±0,4      | 2,8±0,3        | 3,8±0,5        | 3,8±0,5       | 4,2±0,5      | 4,0±0,5        |
| TG                     | 2,2±0,2       | 2,0±0,2       | 1,8±0,3      | 1,9±0,3        | 2,6±0,3        | 2,8±0,3       | 2,4±0,3      | 2,6±0,3        |
| Glucose                | 5,8±0,3       | 5,8±0,3       | 5,8±0,3      | 5,6±0,3        | 5,2±0,5        | 5,1±0,5       | 5,2±0,3      | 5,0±0,3        |

\* - significant difference in dynamics since the beginning of the course ( $p < 0.05$ )

\*\* - significant difference in dynamics since the beginning of the course ( $p < 0.05$ )

## CONCLUSIONS

Having analyzed the above literature [1-14], we can conclude that the use of mare's milk in NAFLD, in particular for NASH patients, is pathogenetically reasoned. Saumal medical properties are due to its rich and balanced composition, which has anti-inflammatory, antibacterial, antioxidant, prebiotic, bifidogenic, immunostimulating and hypocholesterolemic effects. The effect of mare's milk in NASH is demonstrated not only in improving clinical symptoms and biochemical liver indices, but also in normalizing cholesterol metabolism. The latest evidence may become a subject to future research studies.

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