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# Role of Enzymes in Piglets

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## ABSTRACT

*The purpose of this research is to determine the impact of whey on transaminases, which are indicators of liver disease. For this purpose, two groups of piglets were fed with different amounts of whey (ad libidum and controlled quantity) in the period of 45 days. Every 15 days values were tested for ALT, AST and ɣ -GT serum of piglets, and these parameters were examined in vitro with pathological and normal control serum. From several samples of whey investigated total protein and albumen, as minerals Fe, K, Ca and P, using a spectrophotometric method. In piglets fed by whey, a significant reduction is obtained in the level of transaminases ($p < 0.05$) the lowest values and the smallest deviation values were obtained in the group of piglets which were fed ad libidum. The results in vitro show that whey performed inhibition on the activity of these enzymes ALT 10,71%, AST 8,51% and ɣ -GT from 18.16% in pathological serum, in serum with normal values, whey performed inhibition on ALT for 39.33%, AST for 29.08% and ɣ -GT for 39.59%. Whey impacts on the reduction of transaminases and performs inhibition of enzyme activity in the in vitro test.*

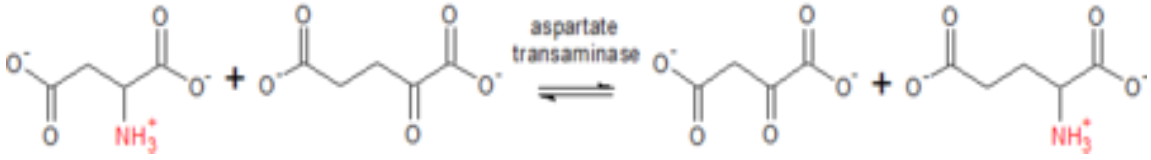
**Keywords:** *ALT, AST, ɣ -GT, piglets, whey, enzymes in vivo, enzymes in vitro.*

## INTRODUCTION

Enzymes are a special class of proteins that catalyze chemical reactions in biological systems, in the human body, animals and plants, a whole range of metabolic processes of decomposition and synthesis takes place (Dzekova, S., 2006). Parameters for testing the function of the liver are the following enzymes: ALT-alanine aminotransferase, AST-aspartate aminotransferase, GT-gamma glutamyl transferase, and others. In the liver, ALT catalyzes the transfer of α-amino nitrogen from alanine to α-ketoglutarate forming pyruvate, which is used in gluconeogenesis (Kaneko, J.J., et al., 2008). ALT is found mainly in the liver, but also in smaller amounts in the kidneys , heart, muscles, and pancreas . ALT was formerly called serum glutamic pyruvic transaminase (SGPT). ALT catalyzes the reversible transfer of the amino group from glutamate to pyruvate while replacing the amino group of glutamate with a carbonyl group, reaction 1.


### Reaction of ALT Catalyzes

AST is found in the liver, heart, skeletal muscle, kidneys, brain, and red blood cells. Serum AST level, serum ALT (alanine transaminase) level, and their ratio (AST/ALT ratio) are commonly measured clinically as biomarkers for liver health.AST formerly was called serum glutamic oxaloacetic transaminase (SGOT).Aspartate transaminase catalyzes the interconversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate. Both ALT and AST levels can test for liver damage, reaction 2.



## Reaction of AST catalyzes

Gamma-glutamyltransferase (also γ-glutamyltransferase, GGT, gamma-GT; EC2.3.2.2) is a transferase (a type of enzyme) that catalyzes the transfer of gamma-glutamyl functional groups from molecules such as glutathione to an acceptor that may be an amino acid, a peptide or water (forming glutamate).This transferase is found in many tissues, the most notable one being the liver, and has significance in medicine as a diagnostic marker increased GGT level indicates that a person's liver is being damaged but does not specifically point to a condition that may be causing the injury. The liver is one of the biggest organs in human body and possesses the remarkable capacity to regenerate. More than 500 vital functions have been identified with the liver. In recent years, the ingredients of milk are functional food because their use has a large impact on health (Marshall, K., 2004). Whey proteins strengthen the immune system, thus helping the body to produce antioxidant glutathione (Marshall, K., 2004). Glutathione protects against free radicals, pollution, toxins and infections. Adding whey protein in the food, can improve the health of people of all ages.

Whey proteins contain all essential amino acids, in higher concentrations compared with some vegetables that are sources of protein such as soy, corn and wheat (Walzem R.L, et al., 2002). Compared with other protein sources, whey has a high concentration of branched chain of amino acids (BCAA) - leucine, isoleucine and valine. BCAA, especially leucine, are very important factors for the growth and regeneration of tissue. Leucine has been identified as a key amino acid in the protein metabolism (Anthony J.C., et al., 2001). Amino acids containing sulfur-cysteine and methionine in whey also present in high concentrations can improve the immune system by intracellular conversion to glutathione (Marshall, K., 2004).

Whey acts as an antioxidant and makes detoxification, due to its participation in the synthesis of glutathione (GSH) which is an intracellular antioxidant. It is the main endogenous antioxidant produced in cells that provides protection of RNA, DNA and proteins through redox cycle of GSH (reduced form) to GSSH (oxidized form) (Bayford, C.L., 2010). Whey is rich incysteine which combines with glutamate and glycine to form glutathione. Riboflavin, niacinamid glutathione reeducates are essential cofactors in the reduction of glutathione (Marz, R., 2010).

The purpose of this research is to examine the impact of whey on transaminases, and thus the function of the liver in pigs, which organic and metabolic function is the same as in the humans. Three groups of pigs are examined, at two groups is added a different amount of whey in the food. The research examined transaminases ALT, AST and ɣ-GT which are indicators of liver damage. The research is done to examine the level of transaminases ALT, AST and ɣ-GT (in vivo) in the blood serum of pigs are fed whey during the 45 days. Examination made of the level of transaminases ALT, AST and ɣ-GT (in vitro) in normal and pathological N P (HUMATROL P and N) control serum with the presence of whey. Examination of the inhibitory activity of the whey on the studied enzymes.

## Material and Methods

The research was conducted on the pig farm. Pigs are fed with protein rich whey which was obtained in technological process of production of mixed cheese (from cow and sheep milk) and cheese. The measurement of transaminases was performed with spectrophotometric methods in biochemistry laboratory using spectrophotometric methods.

Piglets were divided into three groups. One is the control group, which was fed only with concentrate, the second group of pigs despite concentrate, was added a defined amount of whey, while the third group of piglets were fed ad libidum (indefinite quantity of whey). Every two weeks despite normal food, piglets from experimental groups are added whey, which quantity is gradually increasing.

| Days | 0-15 | 15-30 | 30-45 |
|---|---|---|---|
| K group | 0 | 0 | 0 |
| B group | ~2L | ~5L | ~10L |
| A group | ~10L | ~15L | ~20L |

**Table1.** *Amount of whey added to the food of piglets during the 45 days*

All pigs were monitored during 45 days, every 15 days including zero day, blood is taken to examine the amount of serum transaminases to see the change in ALT, AST and ɣ-GT (*in vivo).*During the survey, *in vitro* studies are made with HUMATROL P (with pathological serum values) and N (with normal serum) control serum based on animal serum which was added a quantity of whey, in order to see the inhibitory power of the whey on the transaminases ALT and AST. All parameters are determined spektrophotometricaly, by means of the spectrophotometer (Screen Master) intended for clinical biochemistry. For determination of the activity of ALT and AST, as a recommendation by the International Federation of Clinical Chemistry, kinetic methods are used (Schumann, G. et al, 2002).The results are processed in Microsoft Office Excel and used a test ANOVA. Test ANOVA is the best research method in agronomy, livestock, veterinary and biological sciences and more widely where comparisons are made on more than one factor modalities (Ott, R.L., Longnecker, M., 2001).With the help of this package, tables and graphical representation are done of the survey results.

## Results and Discussion

### Examination of ALT (in Vivo) in Pigs Fed with whey

The results obtained from the examinations on the impact of whey on transaminases in piglets for the concentration of ALT *(in vivo*) are given in Table 2.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **zero day** | **ALT/ (U/l)** | **K** | 7 | 78,67 | 16,87 | 21,45 | | |
| | | **A** | 7 | 87,31 | 21,99 | 25,19 | | |
| | | **B** | 7 | 90,25 | 19,46 | 21,56 | | |
| **15th day** | | **K** | 7 | 85,2 | 19,6 | 23 | | |
| | | **A** | 7 | 77,88 | 9,52 | 12,23 | | |
| | | **B** | 7 | 100,9 | 4,51 | 4,45 | | |
| **30th day** | | **K** | 7 | 92,66 | 33,11 | 35,74 | | |
| | | **A** | 7 | 64,53 | 7,92 | 12,26 | | |
| | | **B** | 7 | 87,59 | 14,56 | 16,63 | | |
| **45th day** | | **K** | 7 | 144,3 | 47,64 | 33,02 | | |
| | | **A** | 7 | 75,96 | 6,68 | 8,81 | | |
| | | **B** | 7 | 103,9 | 21,62 | 20,8 | | |
| | | **statistical parameter** | **n** | **$\bar{x}$** | **SD±** | **CV** | | |

**Table2.** *Value ALT transaminases of the three groups of pigs over 45 days*

The differences that arise in the concentration of ALT in the blood of piglets from the control group and experimental groups A and B are significant and there is a statistical significance between the control and the first experimental group, we believe that adding to pigs the whey in the food, has a favorable impact on health of piglets, given by ALT levels. The dynamics of change in ALT concentration in the blood of piglets during research can best be seen from Figure 1.

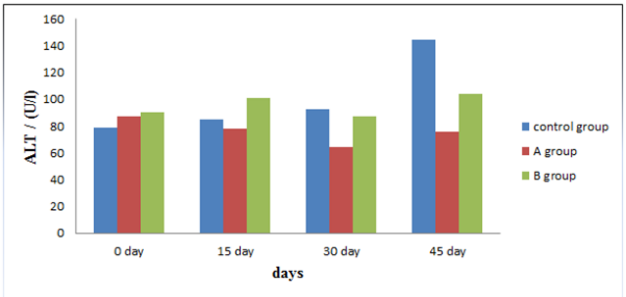



**Figure1.** *Graphical presentation of ALT dynamics in piglets during the examination*

## Examination of AST (in Vivo) in Pigs Fed with whey

The results obtained from studies on the impact of whey on transaminases of piglets, followed by blood levels of AST are given in Table 3. From the data in Table 3 can be seen that the level of AST in the blood of piglets shows some reduction to the 30th day, and then comes to a significant increase, especially in the control group where AST on 45th day reached maximum peak values 142, 46 U / L. As a result of the control group of pigs AST level is the highest, so the existing differences in concentration are statistically significant at the p <0,05 between the control group and the first experimental group A.

| | | statistical parameter | n | $\bar{x}$ | SD± | CV |
|---|---|---|---|---|---|---|
| Zero day | AST/ (U/l) | K | 7 | 55,22 | 5,29 | 9,57 |
| | | A | 7 | 75,63 | 19,33 | 22,56 |
| | | B | 7 | 68,09 | 22,56 | 33,13 |
| 15th day | | K | 7 | 59,06 | 15,05 | 25,48 |
| | | A | 7 | 63,07 | 20,33 | 32,23 |
| | | B | 7 | 67,35 | 8,27 | 12,28 |
| 30th day | | K | 7 | 57,33 | 5,2 | 9,08 |
| | | A | 7 | 54,54 | 23,95 | 43,9 |
| | | B | 7 | 58,84 | 9,32 | 15,84 |
| 45th day | | K | 7 | 142,46 | 60,45 | 42,43 |
| | | A | 7 | 72,26 | 23,28 | 32,21 |
| | | B | 7 | 87,33 | 36,81 | 42,15 |

**Table3.** *Values AST transaminases of the three groups of pigs over 45 days*

The dynamics of change in AST in pigs during the research is shown in Figure 2.

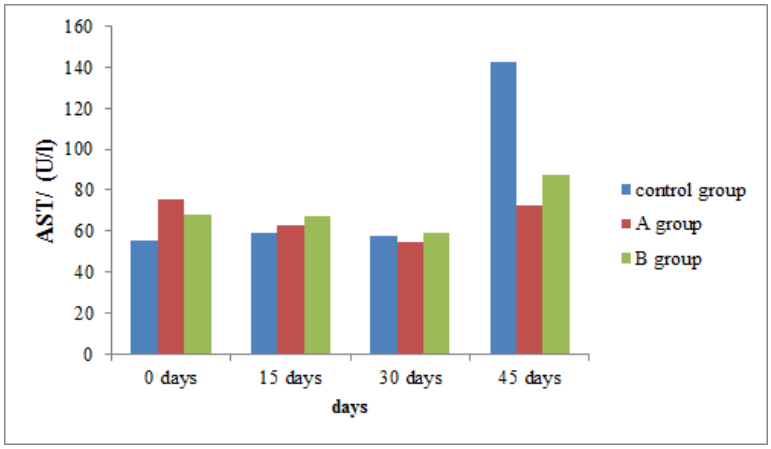



**Figure2.** *Graphical presentation of the AST dynamics in pigs during the research*

## Examining Ɣ-GT (in Vivo) in Pigs Fed with whey

The results obtained from studies on the impact of whey on transaminases or ɣ-GT are given in Table 4.

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Zero day | | | 15th day | | | 30th day | | | 45th day | |
| | | Statistical indicator | K | A | B | K | A | B | K | A | B | K | A | B |
| | ɣ-GT/ (U/l) | n | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| | | $\bar{x}$ | 66,05 | 72,26 | 32,06 | 45,13 | 49,91 | 54,26 | 117,07 | 45,02 | 71,12 | 68,32 | 48,94 | 64,68 |
| | | SD± | 7,67 | 3,23 | 4,4 | 3,92 | 9,19 | 8,54 | 9,28 | 10,75 | 3,31 | 18,57 | 6,22 | 9,64 |
| | | CV | 11,62 | 4,47 | 13,75 | 8,69 | 18,43 | 15,74 | 7,93 | 23,88 | 4,65 | 27,19 | 12,7 | 14,91 |

**Table4.** *Values ɣ-GT of the three groups of pigs over 45 days*

The dynamics of change in ɣ - GT in the experimental period over 45 days can be seen in graph given in chart 3. It can be seen that at the end of the research, the group A has the lowest values, administered *ad libidum*. However, during the research, the lowest values ɣ-GT was obtained on the 30th day and the difference with the control group is the largest.

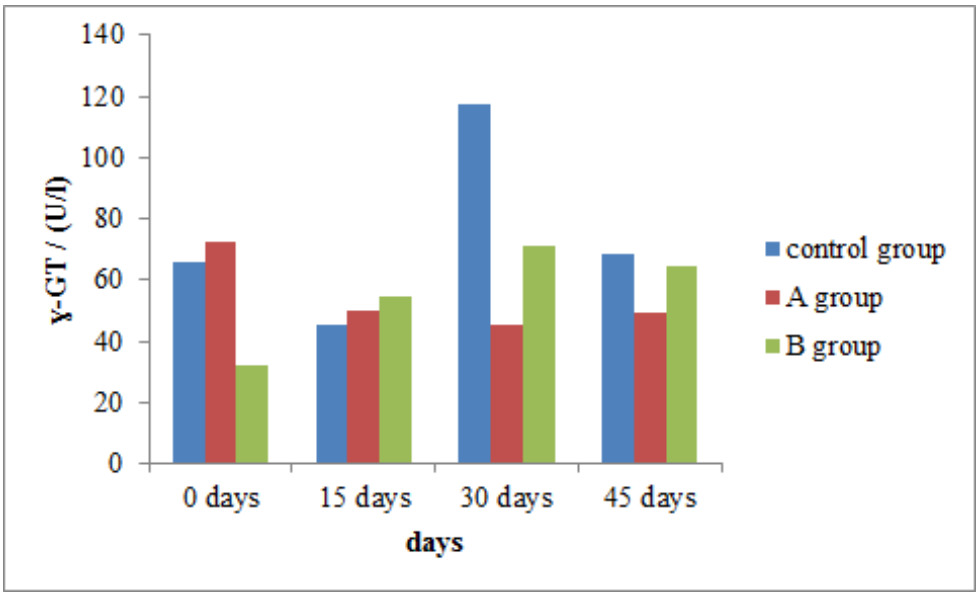



**Figure3.** *Graphic display of ɣ-GT dynamic between the three groups of pigs during the test*

## In Vitro Tests for ALT, AST and Ɣ-GT

In addition to *in vivo, in vitro* effects of whey on transaminases are carried out, in order to see the impact of adding whey in pathological and normal serum. The inhibition of pathological and normal serum (HUMATROL P and N) with whey, obtained results are shown in Table 5 and 6. The results obtained with in vitro studies of the impact of whey on transaminases in normal and pathological serum, we can conclude that whey inhibit the enzyme activity.

| Transaminases | ALT/(U/L) | AST/(U/L) | γ - GT/(U/L) |
|---|---|---|---|
| **Pathological serum-1** | 203,5 | 145,181 | 129,991 |
| Whey-1 | 181,56 | 143,69 | 102,34 |
| **Pathological serum-2** | 186 | 165,368 | 120,153 |
| Whey-2 | 167,43 | 148,56 | 101,04 |
| **Pathological serum-3** | 179,4 | 151,151 | 118,443 |
| Whey-3 | 158,86 | 130,11 | 98,06 |

**Table5.** *Effect of whey on pathological serum*

| Transaminases | ALT/(U/L) | AST/(U/L) | γ - GT/(U/L) |
|---|---|---|---|
| **Normal serum-1** | 50,4 | 46,7 | 48,3 |
| Whey-1 | 31,46 | 39,2 | 29,0 |
| **Normal serum-2** | 48,3 | 45,4 | 45,4 |
| Whey-2 | 28,42 | 26,1 | 27,6 |

**Table6.** *Effect of whey on normal serum*

## Calculating the Inhibitory Activity of Enzymes on whey

The percentage of whey inhibition on transaminases is calculated by a mathematical formula (Kaiser, C., et all.,2007).

**% Inhibition = [(normal activity - inhibited activity) / (normal activity)] * 100%**

It has been received that the whey inhibits the ALT 10,71%, the AST 8,51% and ɣ-GT 18,16% in pathological serum. The serum of normal whey inhibits the ALT 39,33%, the AST 29,08% and ɣ-GT 39,59%. Whey acts as an inhibitor, affecting on activity of ALT, AST and ɣ-

GT, which can be seen from Table 7, their activity is expressed as a percentage. It is evident that both the pathological and the normal serum activity is reduced to about 0, 2%.

**Table7.** *The activity of the enzyme (%)*

| Type of experiment | ALT/ % | AST/ % | ɣ- GT/ % |
|---|---|---|---|
| P | 1,89 | 1,53 | 1,22 |
| P + C | 1,69 | 1,41 | 1 |
| N | 0,49 | 0,46 | 0,47 |
| N + C | 0,29 | 0,33 | 0,28 |

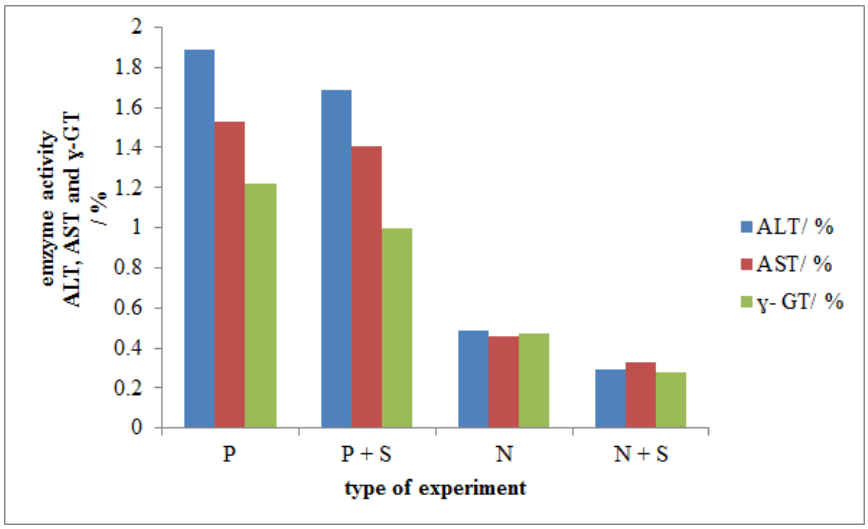



**Figure4.** *The activity of the enzyme (%)*

In Figure 4 enzyme activity (%) can be seen in normal and pathological serum with and without the influence of whey. Scientific data indicates that whey proteins contribute to lower levels of transaminases; therefore in this study we used whey as a food supplement in piglets. The pig esophagus is very similar to that of man, i.e. organic and has the same metabolic function; thereby the healing power of whey on humans can adequately be investigated by feeding pigs with whey. Studies of the enzymes ALT, AST and ɣ-GT of pigs fed with definite and indefinite (*ad libidum)* amount of whey in *vivo*, as well as studies done *in vitro* (using pathological and normal serum) showed a large effect of whey on their activity and has great importance in the piglets food. Our results from this study show that the application of whey in the pigs' food comes to reducing transaminases. The statistical data processing from this study, indicate that on the 15th and 45th day there is a statistically significant difference in the values of ALT among the three groups of pigs, at p <0,05 (Test Anova). The 15th day is a significant difference between groups A and B, and the 45th day between the control group and group A, which is fed *ad* libidum. From table 2, it can be seen that the medium value for the ALT group K is growing throughout the research. The zero day is = 78, 67 U/L, and the 45th day is =144,32. Group A during the research has lower ALT values compared to the control group, and the greatest difference in their values is the 45th day, where ALT is =75,96 U/L. This group of pigs is fed with more than 20 L per day, which means that large quantity of whey in their food affects positively. The group B, which is fed with a controlled amount of whey about 10 L per day, at 7 pigs is noticed an increasing compared to zero day, but it, has significantly lower values compared to the control group at the end of the study. At the zero day, the medium value of group B, ALT is 90, 25 U / L, and on the 45th day is 103, 94 U / L. The lowest values were obtained in Group A on the 30th day (= 64, 53 U / L), when they were fed with 15 L of whey a day for 7 piglets. The same procedure is repeated for AST and data show that on the 45th day, the differences between the control group and group A, fed *ad libidum* are statistically significant at p <0,05 (Box 8). From Table 3, it can be seen that the medium value of AST Group K is growing throughout the study and the 45-th day is rapidly growing which is 142,46 U / L, the zero day is = 55.22 U / L. At the end of the study, there was an increase of AST values among the three groups of pigs, compared to previous days. However, values in groups A and B are reduced; compared to the control group, especially in pigs from group A, fed *ad libidum*. At the zero day, the medium value of group B AST is 68, 09 U / L, at the 45th day 87,33 U / L, but it is significantly lower than in the group K (= 55,22 U / L). The lowest medium value for AST is observed at the 30th day, in group A is 54, 54 U / L, when pigs

are fed with whey about 15 L per day for 7 pigs. The statistical processing of data for ɣ-GT show that there is a significant difference between the groups at zero day and at the 30th day at p <0, 05 (according Anova test). At the zero day there is significant difference between group B with groups K and A at level p <0, 05. At the 30th day there is significant difference between the control group A and B groups. From this it can be concluded that the application of 5-15 L whey daily food, has a positive effect on ɣ-GT. The control group has significantly higher values than the other groups at the end of the study (= 68,32U / L), especially on the 30th day where the medium value is 117, 07 U / L. During the research and lowest values ɣ-GT were obtained on the 30th day in group A (= 45, 02 U / L), and at the end of the research value is slightly higher = 48,94 U / L. This means that adding more than 2 L of whey a day at 7 piglets acts positively on the activity of ɣ-GT, because the values of groups A and B are immediately reduced compared to the control group. What's the influence on whey proteins, the best research has been performed by (Chitapanarux et al., 2009). According to him, providing a daily dose of 20 g protein isolates of whey during 12 weeks to patients with fatty liver, there was a significant ALT and AST reduction. In our research we found a decrease in transaminases after 45 days consuming whey in different amounts. The value of transaminases at the control group fed only with concentrate has been increasing steadily, while the experimental groups A and B were observed consistently reducing the activity of enzymes. The largest reduction after 45 days compared to the control group, has group A, which was administered *ad libidum*. To confirm the positive impact of whey on the activity of ALT, AST and ɣ-GT in the blood of piglets are performed *in vitro* studies, using control serum with normal and pathological values. Based on *in vitro* studies, it was found that whey in those conditions affects on the activity as an inhibitor of these enzymes. The performed calculations showed that whey inhibit the ALT 10,71%, the AST 8,51% and ɣ-GT 18,16% in pathological serum. The serum of normal whey inhibits the ALT 39,33%, the AST 29,08% and ɣ-GT 39,59%. Since we do not know exactly which active ingredients inhibitory effect on trasnaminases, that's why it was examined the composition of used whey or some of the components of whey, if it is possible to find a relationship between the structure and inhibition of enzyme activity. Transaminases are the main indicators of liver disease. Therefore, serial liver hypoxia research was made in the newborn pig-to ALT, AST and LDH in order to predict liver injury because a little it is known about the proportion of the value of different enzymes that are indicators of normal and injured liver (Carlson M., et al., 2009). Alanine aminotransferase (ALT) is an important enzyme, mostly found in the liver. Its increased activity usually occurs because of leaking damaged liver cells. Aspartate aminotransferase (AST) is similar to ALT and its activity is associated with the liver, but it can also be an indicator for the diseases of other organs. Similar studies on the impact of plant proteins were performed with pigs that are fed with mixed feeding with different levels of lysine in the food and the improvement of meat was noticed in the area of the Longissimus dorsi, reducing the average fat thickness. Using the different sources of protein in the diet of pigs, especially adding lysine enables greater increase in muscle mass, or more superficial the Longissimus dorsi, with a larger share of meat, and a smaller share of fat (Marin, M., et al., 2003). Hamad, E., (2011) in their research concluded that oral administration of whey proteins decline in the values of ALT and AST in rats with fatty liver. According toSiddiqui et al. (2008) consuming a greater amount of whey protein, Ca and vitamin D, resulting in reduced accumulation fat and proportionally increasing the expression of insulin receptor, regardless the level of calories of fat or sucrose. Whey, used in our research is rich in Ca, but the third whey has the most 9,5 mmol / l. This study shows that the tested components of whey contain the most expected proteins, especially the first two samples obtained in the cheese manufacture 12 g / L. The research of Wu X., (1998) concluded that combining whey and potassium improves the condition of heart diseases. The whey contains high amounts of K, in our case it has maximum 26,6mmol / l , but not enough to meet the daily needs and further addition of potassium has a positive impact on rehabilitation of heart disease. The results of this research on the impact of whey on transaminase levels in piglets don't give answers which active whey ingredients cause inhibition of enzyme activity. According to numerous literature data presented in the scientific literature assumes that some amino acids are responsible for the enzymes activity, but it leaves room for further research, because controlling liver disease as well as the application of whey is a very important issue.

## CONCLUSION

Based on the performed tests on the impact of whey on piglets, the following conclusions can be made:

1. By adding whey in the pigs' food, the enzyme activity of ALT, AST and ɣ-GT is significantly reduced in the control group;
2. Whey reduces the enzyme activity the most in group A i.e. pigs fed with whey *ad libitum;*
3. The lowest values of ALT, AST and γ-GT are obtained at the 30th day in Group A, the experimental group of pigs ALT = 64,53 U / L, the AST = 54,54 U / L and γ-GT = 45, 02 U / L;
4. From the results can be concluded that the whey affects the enzyme activity in the best way while each pig is given 2 L of whey per day;
5. Whey inhibits during *in vitro* testing of serum with normal and pathological values of ALT, AST and ɣ-GT;
6. At pathological serum, whey inhibits the enzyme activity, like ALT 10,71%, the AST 8,51% and γ-GT of 18,16%;
7. At the serum with normal value, whey inhibits ALT from 39.33% to 29.08% of AST and γ-GT for 39.59%;
8. Whey reduces the activity of serum ALT, AST and ɣ-GT; the increased levels are indicators of liver disease, so whey helps in the treatment of these diseases.

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