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Evaluating the effects of recommending High amount of methionine on anemia in rabbit

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ABSTRACT

The effect of high and extra amount of methionine (%1.2 of total weight of nutrition has been considered on rabbits. 30 rabbits were divided into 2 groups of control (10) and attendance (20). The control were given nutrition without methionine and attendances were given nutrition with high and additional methionine. Taking blood was done once every 10 days. Totally during 10 step it has been done. The samples were tested for anemia. It has been continued for 3 month. The result showed that high and additional amount of methionine with the increase of the amount of homocystein of plasma leads to the damage of tissue. Also it causes mild anemia.

Key words: nutrition, amino acids, methionine, anemia, rabbit

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INTRODUCTION

Nowadays although it has been done some improvement in different field but the problem of nutrition is located at the first grade socially and economically yet and it's as a strategic problem. Metabolic disease is distinctively related to nutrition and has high importance. These diseases have high importance and therefore it has economic damage. So studying these kinds of deficiencies have high importance. Protein and amino acids is one of important elements of nutrition and considering effect of using them incorrectly can be useful for preventing some nutritive deficiencies. One of the important amino acids is methionine that is a part of soulphurate amino acids. Methionine is a necessary amino acids in mammals and birds [1], although the importance of methionine in nutrition of poultry and cows and also about consumption of amino acids in sportsman, a study on high amount of methionine on blood factors has not been done but it is rare. Therefore considering this point for recognition of the effect of high consumption of amino acids with its direct social damages that lead to the economic damages at decreasing weight production is necessary.

MATERIALS AND METHODS

Animal and treatments

30 healthy adult male New Zeland white (NZW) rabbits (*ortycotolagus cunigulus*) , weighing 250-300 g, were provided from the animal laboratory of Tabriz University of Medical Sciences. The experimental animals were randomly divided in two groups (10 in the first group [control group] and 20 in the second group [care group]), then the animals were accommodated individually in stainless metabolic cages under controlled temperature (21-23 ^{0}C) in a 12 hour light/dark cycle with free access to water, labium and pellet food diets. The first group was given rabbit commercial diet contenting alfalfa meal, cornmeal, barley, wheat, soybean meal and minerals as well as vitamins. The other group was given an experimental diet containing 1.2 percent methionine by weight in the diet for 3 months.

Blood sampling

Blood samples were collected from the marginal ear veins of rabbits, then whole blood was collected aseptically using sterile 2ml syringe with 25 gauge needles and poured into tubes without anticoagulant and with anticoagulant. The blood samples without anticoagulant was centrifuged at 3000 g for 10 minutes for room temperature and sera was harvested using disposable micro tubes (Eppendorf, Germany). Serum was analyzed on the same day. This process was repeated in 10 day intervals over a three month period.

Hematological variables [red blood cell count (RBC), hematocrit (Ht), hemoglobin (Hb), mean cell volume (MCV), mean cell hemoglobin(MCH), mean cell hemoglobin concentration (MCHC)] were measured by H1E hematology analyzer (Technicon).

Serum homocysteine level

The serum homocysteine level were determined by Enzyme Immunoassay (EIA) using commercial kits (Axis®-Homocystein EIA, United Kingdom).

Statistical analysis

All data are expressed as mean ± standard error (SE) of the mean. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncans Test; a value of $P<0.05$ was considered statistically.

RESULTS AND DISCUSSION

On the other hand because useful and harmful effect of methionine is similar therefore studying different amount of methionine and its effect is necessary. The result shows that attendance group was fed with nutrition of methionine with additional amount (%0.2 at total weigh of methionine). In comparison to the control group it shows a mild anemia but there isn't a meaningful difference among the number of WBC and plackets among attendance and control groups. The number of RBC and concentration of hemoglobin and the percentage of hematocryte at rabbits of attendance group comparing to control group during first 2 month of studying don't have meaningful difference with control group but at third month the difference among control group and attendance is meaningful. The result of hematology is mentioned in chart 2. It shows that over time the high and additional amount of methionine lead to mild anemia in rabbits of attendance group comparing to control one. This result confirm the result obtained by Sakino et al [2] and Charls et al [3] that shows hemolytic anemia at lat (rabbits 0 that were fed together (5/17). But in this research different amount has been used in comparison to 2 researches and its duration is longer.

The reason that methionine lead to anemia are 1-high and additional amount of methionine that causes deposition and displacement of ferrous at splenalgia, liver, brain and bone and causes anemia, lack of ferrous (microcysteic-Hypocromic) [4].

2-methionine with high amount of intestine absorption halters hystidine because hystidine is a necessary amino acids for making hemoglobin and therefore through disorder in synthesis of globine, hemoglobine can have function in appearance of anemia 3-it is believed that methionine has less poisonous features itself and it is metabolites of methionine that causes its poisonous ness and one of the important metabolites is hemosystein Markus et al [5] showed that Hiperhomocysteimia is a new dangerous factor for Osteoporosis and can decrease the quality of brain and bone and lead to low activity of brain and bone and anemia [6] 4-on the other hand it is showed that there is a relationship among concentration of homocystein in serum and anemia at patients who were homodialized.

It isn't distinguished that what mechanism lead to anemia but this subject that concentration of homocystein can damage Andotelium was proved that causes vessels damage (like disfunction of Andotelial), augmentation of flat multiple cells and tiolation of lipoprotein). Also it affects concentration of placket and blood coagulation. On the other hand Sunilgoomber [10] showed that Homocystinuria is a rare cause of Megaloblastioc anemia that classic Megaloblastioc is related to second Homocystinuria. In this kind of Homocystinuria there is a deficiency in vitamin B_{12} that lead to defective synthesis of DNA that it interfere with metabolism of foulat that active form of foulat that is tetrahitrofoulat will be made that is called 5 and 10 millin tetrahidrofoulat and this form will have function as foulat coenzymes in the reaction of transforming Deoxyuridylate 13 to Tithymidylate 14 that it is used in synthesis of DNA in this way that there is a series of anzymes of Andonucleotidaron that remove oracyled alkalin wherever they exist but it doesn't have any effect on organic alkaline having timidine. While there is lack of foulat, the foulat coenzymes have deficiency and the number of alkaline having oracyls will increase and these Andeonocleotidaz enzyme will remove oracyls alkaline but there isn't organic alkaline having timidine instead. Therefore they will damage the whole structure of nucleus. It hasn't reported many cases of Megaloblastic with first Homocystinuria but pathogen interfering in this event is due to consumption of Militilintetrahidro foulat at mutilation of Homocystein and producing methionine.

Table 1. Levels of hematological variables and serum Homocysteine concentration of rabbits fed with excess high dose of methionine over a Time period

Data are expressed as mean ± SE.

Parameters	Frist group (control group)	Second group (treatment group)								
		10th day	20th day	30th day	40th day	50th day	60th day	70th day	80th day	90th day
RBC, 10^6.mL	5.78±0.072^a	5.48±0.65^a	5.34±0.35^a	5.31±2.66^a	5.49±2.71^a	5.34±1.57^a	5.22±2.92^a	5.1±1.20^a	4.95±1.30^b	4.75±2.24^c
Hb, g.dL	11.65±0.88^a	11.23±0.85^a	11.3±0.15^a	10.63±2.90^a	10.55±2.70^a	10.45±2.27^a	10.35±2.51^a	10.26±2.40^a	10.1±2.60^b	9.95±2.60^c
Ht, %	41.97±0.54^a	40.2±0.89^b	37.96±1.62^c	34.57±2.31^a	38.62±1.28^a	35.81±1.59^a	33.5±1.71^a	33.00±1.99^b	32.33±1.06^b	32.10±1.31^c
MCH, pg	21.97±1.08^a	21.47±0.26^a	21.14±0.46^a	21.37±0.55^a	20.58±1.73^a	19.52±0.47^a	20.38±0.40^a	19.96±1.19^a	19.45±1.20^a	18.85±0.32^b
MCV, fL	73.72±1.28^a	71.76±1.08^a	70.3±1.35^a	68.24±1.45^a	70.33±1.71^a	66.86±1.15^a	65.71±1.47^a	65.13±1.86^a	64.5±1.91^a	64.4±1.36^a
MCHC,g.dl	30.74±2.12^a	30.14±2.45^a	29.72±1.85^a	31.07±2.41^a	29.37±1.75^a	29.26±2.12^a	31.08±1.75^a	29.62±2.02^a	29.34±1.36^a	30.38±1.92^a
PLt, 10^3.mL	398±167^a	402±125^a	398±130^a	366±115^a	372±144^a	365±175^a	403±200^a	389±246^a	410±152^a	385±112^a
WBC, 10^3.mL	8.78±0.42^a	9.31±0.65^a	8.63±2.08^a	8.04±2.35^a	7.5±1.35^a	7.41±1.56^a	7.35±1.78^a	6.95±1.62^a	7.12±1.23^a	7.24±1.54^a
Homocysteine, µmol L^{-1}	0.234±0.11^a	0.48±0.20^a	1.25±0.30^a	2.51±0.54^b	4.47±0.80^c	5.63±0.60^d	7.09±0.85^e	9.34±1.39^f	12.43±1.69^g	14.38±1.33^h

CONCLUSION

Therefore regarding the above subjects, methionine itself has little effect but its metabolites are severely poisonous that the important one is homocystein. Therefore in this research among metabolites of methionine, homocystein is chosen for measuring, that it has increased over time in control group comparing to attendance group meaningfully that in table 1 the result was reported. Therefore we can identify to some extent the reason of changes that is created in factors of hematology that by the increase of hemacystein that its poisonous-ness is proved.

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