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Hepatic Triacylglycerols and Serum Non-Esterified Fatty Acids, Vit. E and Selenium Levels in Cross Breed Cow in Tabirz City of Azarbaijan Province of Iran : An Abattoir Study

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Abstract: The aim of the present study was to compare the hepatic Triacyiglycerols (TAG) and serum Non-esterified Fatty Acids (NEFA) and serum vit. E and selenium in cows in different stages of productivity slaughtered at Tabriz abattoir. So, a total of 204 blood and liver samples were collected from the indigenous hybrid cows immediately after being slaughtered at the abattoir. The cows were divided into 4 groups according to their pregnancy statuses: 1-8 months pregnant, 8-9 months pregnant, less and more than 1 month of parturition. Percentage of TAG were measured in the liver and NEFA, vit. E and selenium were measured in the blood circulation. All the liver samples, in this study, show some degrees of fatty infiltration. The accumulation of that in the liver rose significantly ($p<0.001$) during the 1st month after parturition and returned to the same level of pregnancy after 1 month. We also noticed that serum NEFA concentration was significantly higher ($p<0.001$) during the 1st month of parturition and serum vit. E and selenium level was significantly lower ($p<0.001$) in newly calved group (<1 month after parturition). These results reveal that fatty liver is quite common in all the animals in the region especially in their early lactation. These findings indicate the necessity of reconsideration in the animal's food policies in the area and some especial approaches during the early phase of lactation.

Key words: Cow, fatty liver, pregnancy, malnutrition, parturition, NEFA

INTRODUCTION

Dairy cows undergo tremendous changes during the transition period, i.e., from 3 weeks before to 3 weeks after parturition (Drackley, 1999). This critical important period is characterized by dramatic changes in nutrient demand that necessitate coordinated changes in body tissue metabolism to meet requirements of energy (Bell, 1995; Overton *et al*., 2001). During times of energy deficiency, animals break down triglycerides stored in their adipose tissue and produce Non-esterified Fatty Acids (NEFA) which enter the blood circulation and are transported to organs and tissues throughout the body (Emery *et al*., 1992).

Extensive and prolonged mobilization of body fat leads to fat accumulation in the liver which is called hepatic lipidosis or fatly liver (Gruffat *et al*., 1996; Goff and Horst, 1997). Fatty liver occurs when the hepatic uptake of lipid exceeds the rate of disappearance of triglyceride from the liver through either oxidation or secretion via VLDL (Bremmer *et al*., 2000).

Overfeeding during the non-lactating stage reduces feed intake and stress near patunition accelerates the release of NEFA from adipose tissues, resulting in an excess uptake of NEFA by the liver (Rukkwamsuk *et al*., 1998). Circulating NEFA are absorbed by the liver and re-estrified to Triacylglycerols (TAG) which are then secreted into the blood in the form of VLDL (Herdt *et al*.,1988). Excess hepatic lipids are stored as TAG and are associated with decreased metabolic functions of the liver (Drackley, 1999). In dairy cows, fatty liver occurs primarily in the 1st month of lactation (Grummer, 1993) when up to 50% of all cows have some accumulation of TAG in liver (Jorritsma *et al*., 2001). Approximately, severe and moderated fatty liver develop in 15 and 35% of the dairy cows, respectively (Rehage *et al*., 2006).

Severity of fatly liver can be defined on the basis of amount of TAG accumulation. Bobe *et al*. (2004) categorized fatty liver into normal (<1% liver TAG on wet weight, bases), mild (1-5% liver TAG), moderate (5-10% liver TAG) and severe (>10% liver TAG). Although, fatty liver is associated with increased incidence of health disorders (Jorritsma *et al*., 2000). Recent studies have showed no relationship between liver function and the liver's fat content (Rehage *et al*., 2006). It is believed that the majority of cows even those with severe fatty liver. May not reveal impaired liver function in the blood biochemistry tests (Rehage *et al*., 2006) therefore, liver biopsy is the only reliable method to determine severity of fatty liver in the dairy cows. The objectives of this study were to assess the percentage and severity of fatty liver among cross breed cows in Tabriz in the east of Iran. An attempt was made to confirm the previous mentioned problem by measuring TAG in the liver and NEFA, vit. E and selenium concentration in the blood circulation.

MATERIALS AND METHODS

The research was carried out in Tabriz, the capital of Azarbaiejan province which is placed in the 600 km to Teheran, the country's capital. During a period of 3 months from the beginning of July to the end of September 2010, blood and liver samples were collected from 204 cross breed cows (Holsteinxindigenous) at Tabriz's abattoir and were classified into 4 groups regarding the cow's pregnancy status: 1-8 months pregnant, 8-9 months pregnant, less and more than 1 month of parturition.

The cows were sent to the pastures with inferior quality grasses during the day and in their return to their barns they were nursed with 2-3 kg of a locally made concentrate containing barley, wheat and maize. The cows had access to ad lib straw. The daily milk production of these cows was 16-20 kg day^{-1}. Blood was taken for the determination of NEFA, vit. E and selenium while the calves were being slaughtered. After collection, the blood samples were allowed to stand for 20-30 min and then they were transferred to the laboratory where they were centrifuged at 2000-3000 rmp.

The serum was separated and stored at -20°C till analysis. Liver samples of at least 10 g weight were taken from a certain area of the right lobe after the animal's abdomen was opened. For TAG determination, the liver samples were placed in physiological saline and carried to the laboratory in a thermostatic (0-4°C) container. Lipid extraction from the liver carried out by the method of Folch *et al.* (1957). TAG concentrations were measured as described by Neri and Firngs (1973) and Frings *et al.* (1972), respectively.

Serum vit. E and selenium concentrations were measured as explained by HPLC and atomic absorbtion spectrophotometery, respectively. Serum NEFA level was determined using also spectrophotometer with a commercially available Kit (Randox). Analysis of variances and post-hoc Tukey's honestly significant difference tests were used to analyze the data. All statistical analyses were performed using the SPSS software, version 13.0. Significance was declared at $p<0.001$.

RESULTS AND DISCUSSION

As it is shown in Table 1 in newly calved group (<1 month after parturition), the percentage of TAG accumulation in the liver was significantly ($p<0.001$) higher than other 3 groups. Mean serum NEFA concentrations were also significantly ($p<0.001$) higher for cows with <1 month of parturition. Mean concentration of vit. E was significantly lower ($p<0.001$) for cows with <1 month of parturition. Also mean concentration of selenium was significantly lower ($p<0.001$) for cows with <1 month of parturition. The prevalence of moderate as well as severe fatty liver in this cohort of 204 cows was 16.2% (Table 2). The total percentage of cows with mild fatty liver was 65.5%, the moderate group 15.5% and the cows with severe fatty liver composed 0.7% of the animals.

Based on the Bobe *et al.* (2004), classification about 44.3, 51.3 and 3.4% of the cows in the present study had mild, moderate and severe fatty liver, respectively. Reid (1980) reported that the incidence of moderate and severe fatty liver in Holstein cows in England was 48 and 15%, respectively and in Guernsey cows, he showed a 33% moderate and 5% severe fatty liver. In the USA, incidences of 20% for moderate and 15% (Gerloff *et al.*, 1986) to 24% (Herdt, 1991) for severe fatty liver were reported. In a review of the literature, Bobe *et al.* (2004) indicated that in the 1st month after calving, 5-10% of dairy cows show severe and 30-40% show moderate fatty liver which means that up to 50% of dairy cows are at a higher risk of the disease. In present study, we noticed that about 90.9 and 4.6% of the indigenous dairy cows had moderate and severe fatty liver, respectively in the 1st month after parturition. This can be due to the nutritional mismanagement during dry period and stressful condition after parturition. In contrast to the Reazai Saber *et al.* (2007) and Gerloff *et al.* (1986) reports who showed that severe fatty liver occurred prior to parturition, we in line with the finding of Van den Top *et al.* (1996) observed that severe fatty liver mainly developed in the 1st month after calving.

It has been shown that postpartum accumulation of TAG in the liver is a possible consequence of a postpartum negative energy balance which originates from a reduction or diminished pre-parturient dry matter intake and higher demands of energy due to the start of milk production (Bertics and Grummer, 1999). In these conditions, NEFA are mobilized from the adipose tissues and transported to the liver where some are re-estrified to TAG which are then secreted into the blood circulation in the form of VLDL (Mazur *et al.*, 1989). However when the intra-hepatic NEFA concentrations increase, the

Table 1: Percentage of TAG in the liver and the levels of serum NEFA, vit. E and selenium

Groups	TAG (%)	vit. E ($\mu g\ dL^{-1}$)	NEFA (μ Eq L^{-1})	Selenium ($\mu g\ mL^{-1}$)
During 1st month of parturition	8.07±1.46*	145.09±15.09*	1332.50±157.61*	0.047±0.02*
After 1st month of parturition	1.20±0.75	199.34±21.97	612.74±123.00	0.148±0.04
1-8 months pregnant	2.40±0.31	190.66±14.28	711.74±76.690	0.119±0.03
8-9 months pregnant	3.36±1.27	180.51±12.15	821.02±175.30	0.109±0.03
Total	3.15±0.45	183.73±24.43	804.92±273.24	0.114±0.04

*p<0.001 within the column

Table 2: Prevalence of different grades of fatty liver changes among the studied groups

Groups	No. of cases	Normal liver	Mild fatty changes	Moderate fatty changes	Severe fatty changes
		(Percentage of cases)			
Before 1st month of parturition	32	0.0	4.5	90.9	4.6
After lst month of parturition	62	60.5	39.5	0.0	0.0
1-8 months of pregnant	60	0.0	100.0	0.0	0.0
8-9 months pregnant	50	0.0	94.3	5.7	0.0
Total	204	18.3	65.5	15.5	0.7

production of VLDL is impaired and the liver accumulates a great amount of TAG. It has been shown that (Grummer, 1993) the concentrations of NEFA increase gradually during the final weeks prior to parturition and sharply elevates at parturition however, the greatest increase was observed between 1-3 weeks after parturition (Rukkwamsuk *et al*., 1998). It is believed that the rapid rise in NEFA at parturition could be due to the stress of calving. serum NEFA decrease after calving but remain higher than what they were before parturition (Grummer, 1995).

In present study, a significant increase (p<0.001) in serum NEFA was observed in the newly calved cows which was in accordance with the finding of Reid *et al.* (1983) who showed that plasma NEFA concentrations 1 week after parturition were approximately twice as high in cows with moderate fatty liver and they concluded that the increased serum NEFA could be responsible for fatty liver. In present study, we noticed 4.6% of the recently calved cows had severe fatty liver with concomitant high serum NEFA concentrations.

These results are in accordance with the finding of Gerloff *et al.* (1986) who showed that cattle with severe hepatic lipidosis had the greatest serum NEFA. A high serum NEFA is commonly seen during the negative energy balance of post partum dairy cows due to the shortage of energy and subsequent body fat mobilization (Jorritsma *et al*., 2001).

In the current study, the cows during their dry period were fed mostly straw ad lib and small amount of wilted grass and after calving, a mixture of 2-4 kg of barley, bran and dried bread were added to the straw feeding. It has been reported that both over-conditioning and underfeeding of cows during late pregnancy could severely affect the animal productivity and may cause perparturient metabolic disorders (Forbes, 1986; Bell, 1995; Tesfa *et al*., 1999). Gerloff and Herdt (1984) indicated that feeding only straw for 5 days at late pregnancy induced prepartal fatty liver. In present study, we noticed that about 100% cows in their late pregnancy had a mild fatty liver (>1% liver TAG). We believe that the reason for high percentage of fatty liver among the studied cows was a long period of feed restriction before their parturition and feeding them with poor quality foods after their calving. One of the numerous changes may occur in fatty liver is a drop in serum vit. E and selenium (Bobe *et al*., 2004) therefore, serum vit. E and selenium could be used as a marker of the liver's function. Vitamin E blood levels which reflect the dietary vit. E intake for cows (Maplesden *et al*., 1960; Tikriti, 1969) where significantly lower in 1st month after calving as compared to another groups (p<0.001).

In fatty liver syndrome cows, until the 2nd week after calving, blood vit. E levels were lower than those levels reported as normal (Bayfield and Mylrea, 1969; Mukhtar, 1996; Hidroglou and Hartin, 1982; LeBlanc *et al*., 2004; Tappel, 1962). Blood vit. E functions as a source for tissue vit. E and the liver is the major storage or metabolic site of this vitamin. In the liver, many oxidation-reduction reactions require vit. E as a biological antioxidant (Tappel, 1962; Mudron *et al*., 1999). Excess accumulation of fat in parenchymal cells could be related to some interference with the process of fatty acid oxidation or with the synthesis or the intracellular transport of lipoproteins. Vitamin E has a function in promoting the stability and integrity of the lipoprotein membrance cells (Diplock, 1981; Mudron *et al*., 1997). The criteria of successful treatment of fatty liver syndrome cases in which liver dysfunction is thought to be present because of excessive hepatic lipogenesis are rapid reduction of fatty infiltration of liver tissues and restoration of normal metabolism. It was observed (Harrill *et al*., 1965; Prodouz and Navari, 1975; Spratt and Kratzing, 1971) that in laboratory animals fatty liver of diverse origin are alleviated by administration of vit. E. In the present study, the observation of lower blood vit. E in the 1st month after calving (Table 1) suggests a possible cause-effect relationship between fatty liver syndrome and vit. E status of cows and encourages further evaluations of the possible preventive administration of massive doses of vit. E for alleviation of the fatty liver in the periparturient cows. In present study, a significant decrease ($p<0.001$) in serum selenium concentration was observed in the newly calved cows which was in accordance with the findings of Bobe *et al.* (2004), Hidroglou and Hartin (1982) and West (1990).

It was reported (Backall and Scholz, 1979) that difficulties arise when attempting to predict the magnitude of Se inadequacy in dairy cattle which could result in clinical manifestations of Se deficiency. It was found (Perry *et al.*, 1976) that plasma Se levels <20 ng mL^{-1} which is equivalent to at least 56 ng mL^{-1} of whole blood are indicative of Se deficiency in dairy cattle. However, a problem of retained placentas is reported in dairy herds with Se levels of 61-73 ng mL^{-1} of whole blood (Trinder *et al.*, 1973). Serum selenium concentration, shown in Table 2 was suggestive of relation with Se responsive disease in the dairy cow (Conrad *et al.*, 1976; Julien *et al.*, 1976)

CONCLUSION

Results from the present study showed that fatty liver existed either in pregnant or non-pregnant cows and in the 1st 4 weeks after calving was severe than any other times. The researchers believe poor quality of food can be a major factor in this high percentage of fat infiltration in the liver. The high frequency of hepatic lipidosis that was found in the present study may have a great impact on health and fertility of the cows, since it has been shown that even mild fatty liver can be associated with decreased health status and fertility of dairy cows. To reduce fatty liver incidence in cows in Tabriz, it is suggested to supply sufficient nutrients before and after calving.

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REFERENCES

Backall, K.A. and R.W. Scholz, 1979. Reference values for a field test to estimate inadequate glutathione peroxidase activity and selenium status in the blood of cattle. Am. J. Vet. Res., 40: 733-738.

Bayfield, R.F. and P.J. Mylrea, 1969. Carotenoid and tocoperhol levels in the serum of apparently healthy dairy cattle. J. Dairy Res., 36: 137-144.

Bell, A.W., 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim. Sci., 73: 2804-2819.

Bertics, S.J. and R.R. Grummer, 1999. Effect of fat and methionine hydroxyl analog on prevention or alleviation of fatty liver induced by feed restriction. J. Dairy Sci., 82: 2731-2736.

Bobe, G., J.W. Young and D.C. Beitz, 2004. Pathology, etiology, prevention and treatment of fatty liver in dairy cows. J. Dairy Sci., 87: 3105-3124.

Bremmer, D.R., S.J. Bertics, S.A. Besong and R.R. Grummer, 2000. Changes in hepatic microsomal triglyceride transfer protein and triglyceride in periparturient dairy cattle. J. Dairy Sci., 83: 2252-2260.

Conrad, H.R., W.E. Julien and A.L. Moxon, 1976. Plasma selenium levels in supplemented and selenium deficient dairy cows. Distillers Feed Res. Counc. Proc., 31: 49-52.

Diplock, A.T., 1981. Metabolic and functional defects in selenium deficiency. Philos. Trans. R. Soc. London Ser. B Biol. Sci., 294: 105-117.

Drackley, J.K., 1999. Biology of dairy cows during the transition period: The final frontier. J. Dairy Sci., 82: 2259-2273.

Emery, R.S., J.S. Liesman and T.H. Herdt, 1992. Metabolism of long chain fatty acids by rmninant liver. J. Nutr., 122: 832-837.

Folch, J., M. Lee and G.H.S. Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226: 497-509.

Forbes, J.M., 1986. The Effects of Sex Hormones, Pregnancy and Lactation on Digestion, Metabolism and Voluntary Food Intake. In: Control of Digestion and Metabolism in Ruminants, Miligan, L.P., W.L. Grovum and A. Dobson (Eds.). Prentice-Hall, Englewood Cliffs, New Jersey, pp: 420-435.

Frings, C.S., T.W. Fendely, R.T. Dunn and C.A. Queen, 1972. Improved determination of total serum lipids by the sulfo-phosphovanilin reaction. J. Clin. Chem., 18: 673-674.

Gerloff, B.J. and T.H. Herdt, 1984. Hepatic lipdosis from dietaiy restriction in nonlactating cows. J. Am. Vet. Med. Assoc., 185: 223-224.

Gerloff, B.J., T.H. Herdt and R.S. Emery, 1986. Relationship of hepatic lipidosis to health and performance in dairy cattle. J. Am. Vet. Med. Assoc., 188: 845-850.

Goff, J.P. and R.L. Horst, 1997. Physiological changes parturition and their relationship to metabolic disorders. J. Dairy Sci., 80: 1260-1268.

Gruffat, D., D. Durand, B. Graulet and D. Bauchart, 1996. Regulation of VLDL synthesis and secretion of the liver. Reprod. Nutr. Dev., 36: 375-389.

Grummer, R.R., 1993. Etiology of lipid-related metabolic disorders in periparturient dairy cows. J. Dairy Sci., 76: 3882-3896.

Grummer, R.R., 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. Anim. Sci., 73: 2820-2833.

Harrill, I., G. Minarik and E.D. Gifford, 1965. Effect of vitamins A and E on lipids in selected rat tissues. J. Nutr., 87: 424-428.

Herdt, T.H., 1991. Relationship of fat metabolism to health and perfonnance in dairy cattle. Bovine Pract., 26: 92-95.

Herdt, T.H., T. Wensing, H.P. Haagsman, L.M.G. van Gold and H.J. Breukink, 1988. Hepatic triacylglycerol synthesis during a period of fatty liver development in sheep. Anim. Sci., 66: 1997-2013.

Hidroglou, M. and K.E. Hartin, 1982. Vitamins A, E and selenium blood levels in the fat cow syndrome. Can. Vet. J., 23: 255-258.

Jorritsma, R., H. Jorritsma, Y.H. Schukken and H.H. Wentink, 2000. Relationships between fatty liver and fertility and some periparturient diseases in commercial Dutch dairy herds. Theriogenology, 54: 1065-1074.

Jorritsma, R., H. Jorritsma, Y.H. Schukken, P.C. Bartlet, T. Wensing and G.H. Wenting, 2001. Prevalence and indicators of postpartum fatty infiltration of the liver in nine commercial dairy herds in the Lives. Prod. Sci., 68: 53-60.

Julien, W.E., H.R. Conrad, J.E. Jones and A.L. Moxon, 1976. Selenium and vitamin E and incidence of retained placenta in parturient dairy cows. J. Dairy Sci., 59: 1954-1959.

LeBlanc, S.J., T.H. Herdt, W.M. Seymour, T.F. Duffield and K.E. Leslie, 2004. Peripartum serum vitamin E, retinol and beta-carotene in dairy cattle and their associations with disease. J. Dairy Sci., 87: 609-619.

Maplesden, D.C., J.D. Harvey and H.D. Brannon, 1960. Blood plasma tocopherol and phosphorus levels in a herd of beef cattle. J. Nutr., 71: 77-84.

Mazur, A., E. Marcos and Y. Rayssiguier, 1989. Plasma lipoproteins in dairy cows with naturally occurring severe fatty liver: Evidence of alteration in the distribution of apo A-I-containing lipoproteins. Lipids, 24: 805-811.

Mudron, P., J. Rehage, H.P. Sallmann, M. Mertens, H. Scholz and G. Kovac, 1997. Plasma and liver á-tocopherol in dairy cows with left abomasal displacement and fatty liver. Zentralbi Vet. A, 44: 91-97.

Mudron, P., J. Rehage, K. Qualmann, H.P. Sallman and H. Scholz, 1999. A study of lipid peroxidation and vitamin E in dairy cows with hepatic insufficiency. J. Vet. Med. A, 46: 219-224.

Mukhtar, A.M.S., 1996. Some interrelationships between vitamin E and vitamin A in the nutrition of ruminants. Ph.D. Thesis, Iowa State University.

Neri, P. and C.S. Firngs, 1973. Improved method for determination of triglycerides in serum. J. Clin. Chem., 19: 1201-1203.

Overton, T.R., G. Bernal-Santos, Perfield, II, J.W. and D.E. Bauman, 2001. Effects of feeding Conjugated Linoleic fatty Acids (CLA) on metabolism and performance of transition dairy cows. Cornell Nutr. Conf., 63: 179-187.

Perry, T.W., D.M. Caldwell and R.C. Peter, 1976. Selenium content of feeds and effect of dietary selenium on hair and blood serum. J. Dairy Sci., 59: 760-763.

Prodouz, K.N. and R.M. Navari, 1975. Effects of vitamin A and E on rat tissue lipids. Nutr. Rep. Int., 11: 17-28.

Reazai Saber, A.P., M. Nouri, A. Shahriari, A. Rasouli and R. Fatemi Tabatabi, 2007. Hepatic triacylglycerols and plasma non-esterified fatty acids and albumin levels in cross breed cows in Ahvaz city of Khuzestan province of Iran: An abattoir study. Pak. J. Biol. Sci., 10: 2940-2944.

Rehage, J., A. Starke, M. Holtershinken and M. Kaske, 2006. Hepatic lipidosis. Diagnostic tools and individual and herd risk factors. Congress, XXIV world Buiatrics, pp: 69-74.

Reid, I.M., 1980. Incidence and severity of fatty liver in dairy cows. Vet. Rec., 107: 281-284.

Reid, I.M., S. Dew, R. Collins, M. Ducker, G. Bloomfield and S. Morant, 1983. The relationship between fatty liver and fertility in dairy cows: A farm investigation. J. Agric. Sci., 101: 499-502.

Rukkwamsuk, T., T. Wensing and M.J.H. Geelen, 1998. Effect of overfeeding during the dry period on regulation of adipose tissue metabolism in dairy cows during the periparturient period. J. Dairy Sci., 81: 2904-2911.

Spratt, M.G. and C.C. Kratzing, 1971. The effect of dietary lipids and alpha-tocopherol on RES activity in choline deficiency. Res and fatty livers. J. Reticuloendothel Soc., 10: 319-329.

Tappel, A.L., 1962. Vitamin E as the biological lipid antioxidant. Vitamins Hormones, 20: 493-510.

Tesfa, A.T., M. Turoi, L. Syrjala-Qvist, R. Poso and H. Saloniemi *et al*., 1999. The influence of dry period feeding on liver fat and postparthm performance of dairy cows. Anim. Feed Sci. Tech., 76: 275-295.

Tikriti, H.H., 1969. The metabolism of vitamin E by the lactating diary cow in relation to oxidized flavor in milk. Ph.D. Thesis, University of Maryland.

Trinder, N., R.J. Hall and C.P. Renton, 1973. The relationship between the intake of selenium and vitamin E on the incidence of retained placenta in dairy cows. Veterinary Rec., 93: 641-644.

Van den Top, A.M., M.J.H. Geelen, T. Wensing, G.H. Wentink, A.T. van T'Klooster and A.C. Beynen, 1996. Higher postpartum hepatic triacylglycerol concentrations in dairy cows with free rather than restricted access to feed during the dry period are associated with lower activities of hepatic glycerolphosphate acyltransferase. J. Nutr., 126: 76-85.

West, H.J., 1990. Effect on liver function of acetonaemia and the fat cow syndrome in cattle. Res. Vet. Sci., 48: 221-227.