Journal of Elementology ISSN 1644-2296

Zwierzchowski G., Miciński J., Pogorzelska J., Siwicki A., Wójcik R., Kobzhassarov T.Z., Bermagambetova N., Shaikamal G.I., Fijałkowska M. 2016. *Influence of A diet containing β-carotene and omega-3 fatty acids on the biochemical and nonspecific humoral immunity indicators and on the results of experimental calf rearing*. J. Elem., 21(1): 283-302. DOI: 10.5601/jelem.2015.20.1.913

INFLUENCE OF A DIET CONTAINING *β*-CAROTENE AND OMEGA-3 FATTY ACIDS ON THE BIOCHEMICAL AND NONSPECIFIC HUMORAL IMMUNITY INDICATORS AND ON THE RESULTS OF EXPERIMENTAL CALF REARING

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Abstract

The aim of this study has been to determine the effect of a diet containing *β*-carotene and omega-3 fatty acids on the biochemical and nonspecific humoral immunity indicators and on the results of rearing calves to day 60 of life. The study was conducted in 2011-2012, on a herd of Polish Holstein-Friesian cows. 54 calves from the herd were divided into 3 groups, 18 animals in each: CTR, TRT1 and TRT2. During the colostral period lasting for 5 days after birth, calves were drinking the mother's colostrum. From 6 to 60 days, calves were watered milk replacer formulation. The control group (CTR) consisted of calves after the colostral period given milk replacer formulation without any supplementation. In the two other groups, calves received milk replacers with the supplementation of *β*-carotene (TRT1 group) in the amount of 25 mg/calf/day or a suspension of liver omega-3 oil (TRT2 group) in the amount of 5 g/calf/day. All calves received solid feed *ad libitum*. The research showed that the parameters of the metabolic profile of calves (*ALT, ASP, CHOL, GLU, UREA, ALP, TG, CRE*) were within the normal range, indicating good health of the calves. Moreover, the supplementation had a positive impact on the chosen immunological parameters, including a higher IgG concentration, especially at the end of the milk drinking period. The

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result of β-carotene in a diet was the highest daily gain in the period from 30 to 60 days of life among the analysed groups of calves. No such effect was achieved in the TRT2 group of calves, as their body weight was the lowest. However, noteworthy is the beneficial impact of beta-carotene and omega-3 oil on the non-specific humoral immunity parameters, which was manifested by fewer cases of clinical diarrhoea and upper respiratory tract diseases.

Keywords: immunoglobuliny, β-karoten, omega-3 fatty acids, calves, biochemical indicators

INTRODUCTION

Calves are hypogammaglobulinemic immediately after birth and the only immunity they gain comes from colostrum. More recent approaches to calf rearing assume minimising the duration of the colostrum-feeding period. However, potential mistakes made by handling staff or individual predispositions of calves may lead to failure of passive transfer (FPT). The lack of immunoglobulins makes calves prone to illnesses in the first weeks of life, which can negatively affect their future productivity performance. Moreover, health problems can cause deaths among calves, with subsequent economic losses and inferior profitability of milk or beef production.

Calves can grow and develop properly in the absence of infections. Diarrhoea and respiratory infections are the most common causes of health problems, while intestinal or pulmonary inflammation is the most common cause of death. Diseases contracted by calves may affect the future fertility and productivity of the animals. This issue gains importance when young dairy heifers are reared (Faber et al. 2005, Bartels et al. 2010).

Various dietary supplements are used for the prevention of digestive system diseases among calves. Probiotics, prebiotics and synbiotics play a crucial role in stabilising the functions of mucous membranes and inhibiting colonization of the digestive tract by undesirable gastrointestinal microflora or microfauna. Some of these supplements contain polyunsaturated Omega-3 fatty acids (*FA*), of which the most important are *EPA* (*Eicosapentaenoic acid*) and *DHA* (*Docosahexaenoic acid*). These acids are most common in oils derived from marine fish, mainly cod liver oil, or from vegetables (but only *EPA* acid). Research results indicate that these acids, by influencing lipid metabolism, have a favorable impact on the health of calves, their immunity and rearing outcome (Ballou, Depeters 2008, Hill et al. 2009).

According to Kume and Toharmat (2001), and Nishiyama et al. (2011), deaths of calves in the first week of life can be caused by vitamin deficiency, especially the lack of vitamin A, as neonates have very small amounts of this vitamin stored in the liver. Therefore it is highly recommended to feed calves enough volume of colostrum, which is a natural source of this compound and its natural precursor, β-carotene (βc). This powerful antioxidant, which plays an important role in developing immune functions in young calves, is absorbed by intestinal mucosa. It has been shown that carotenoids also protect

lipid membranes from peroxidation or/and oxidation of their unsaturated fatty acids in cell membranes. Some earlier investigations have demonstrated that deficiency of βc can cause diarrhoea in the first period of a calf's life (Michal et al. 1994, Kume, Toharmat 2001, Kaewlamun et al. 2011).

The aim of this study has been to determine the effect of a diet containing β-carotene or omega-3 fatty acids on the biochemical and nonspecific humoral immunity indicators as well as on the results of experimental calf rearing.

MATERIAL AND METHODS

In 2011-2013, a study was conducted on a high-performance herd of Polish Holstein-Friesian cows. Using the method of analogues, 54 calved cows from the 1st, 2nd and 3rd lactation were chosen, from which 54 calves were obtained to be examined afterwards. In the colostral period lasting for 5 days after birth, calves were drinking mother's colostrum. Colostrum was administered to calves in the amount of 2 kg during the first hour after calving and then after every 8 hours.

Throughout a 60-day watering period, calves were kept in individual wooden booths outside the barn and afterwards they were placed in collective pens, called a nursery. During the watering period, the milk replacer made by Polass S.A. Bydgoszcz, Poland, and solid feed were given (Table 1). The

Table 1

Nutrient composition of milk replacer and calf starter fed to all calves*

Nutrients	(% DM)
Dry matter (DM)	94.0
Protein (CP)	21.0
Fat (F)	18.0
Ash (ASH)	8.0
Fibre (ADF and NDF)	0.4
Lactose (LAC)	45.0
Immunoglobulins (Ig)	0.8
α-tokoferol (α-T)	0.2
Amino acids (% DM)**	0.3
Mineral and vitamin mix (% DM) ***,****	0.3

* of 1 kg of milk powder as reported by manufacturer (Polmass SA, Bydgoszcz, Poland);
** 1.74 g lys kg^{-1}; 0.38 g meth kg^{-1};
*** 1.00 g Ca kg^{-1}; 0.65 g P kg^{-1}; 0.60 g Na kg^{-1}; 0.08 g Mn kg^{-1}; 0.07 g Zn kg^{-1}; 0.02 g Cu kg^{-1}; 0.10 g Fe kg^{-1}; 0.01 g Co kg^{-1}; 0.01 g I kg^{-1}, 0.01 g Se kg^{-1};
**** 4k IU vitamin D_3 kg^{-1}; 200.0 mg vitamin E kg^{-1}; 4.0 mg menadione kg^{-1}; 6.0 mg thiamine kg^{-1}; 10.0 mg riboflavin kg^{-1}; 20.0 mg niacin kg^{-1}; 20.0 mg panthotenic acid kg^{-1}; 6.0 mg pyridoxine kg^{-1}; 0.48 mg folic acid kg^{-1}; 0.05 mg cobalamine kg^{-1}; 0.10 mg biotin kg^{-1}

feed contained the following ingredients: calf starter (KCJ), corn silage, triticale meal, hay, mineral and vitamin mixture. The composition of the rations used in period from 6 to 60 day of life is specified in Table 2.

Table 2

The composition of rations calves at different times of watering

Feed	Days of life		
	6-10	11-30	31-60
Milk replacer *, ltrs	6.0	5.0	4.0
Calf starter KCJ **	-	ad libitum	1.0
Triticale meal	-	ad libitum	1.0
Straw	-	ad libitum	0.5
Corn silage (30-35% dry matter)	-	-	0.5
DM (%)	-	42.8	47.2
Ash (% of DM)	-	5.3	5.2
ADF (% of DM)	-	17.3	18.1
NDF (% of DM)	-	33.7	35.1
CP (% of DM)	-	17.4	16.8
Fat (% of DM)	-	4.1	3.7
DE (MJ kg^{-1} of DM)	-	9.0	9.4

* Milk replacer was enriched: 50 mg kg^{-1} of β-carotene in TRT1 or 5 g day^{-1} calf of Omega-3 fatty acids in group TRT2.
The composition of minerals and vitamins milk replacer was as follows: 156.0 g Ca kg^{-1}, 51.0 g P kg^{-1}, 75.0 g Na kg^{-1}, 32.0 g M kg^{-1}, 3.45 g Zn kg^{-1}, 1.36 Mn kg^{-1}, 0.45 Cu kg^{-1}, 0.01 Co kg^{-1}, 0.04 g I kg^{-1}, 0.02 g Se kg^{-1}, 900 k vitamin A kg^{-1}, 90 k vitamin D_3 kg^{-1}, 1.50 g vitamin E kg^{-1}, 0.28 g thiamine kg^{-1}, 0.12 g riboflavin kg^{-1}, 0.10 g pyridoxal kg^{-1}, 0.63 g niacin kg^{-1}, 0.27 g pantothenic acid kg^{-1}, 0.02 g folic acid kg^{-1}, 1.00 mg cobalamin kg^{-1}, 2.00 mg biotin kg^{-1}, 2.00 g phytobiotic (Blattin, Dormagen, Germany).

** Commercial starter mixture contained as reported by the manufacturer: grain meal (oats, whey, barley); soy meal, sunflower grain, rapeseed meal, feed yeasts, plant oil (Polmass SA, Bydgoszcz, Poland).
The composition of minerals and vitamins calf starter KCJ was as follows: 2 5.0 g P kg^{-1}, 2.0 g Na kg^{-1}, 8.0 g Mg kg, 0.14 g Mn kg^{-1}, 0.28 g Zn kg^{-1}, 0.22 g Fe kg^{-1}, 0.48 g Cu kg^{-1}, 0.95 g Co kg^{-1}, 0.03 g I kg^{-1}, 0.21 g Se kg^{-1}, 30k IU vitamin A (retinol) kg^{-1}, 3.1 k vitamin D_3 kg^{-1}, 40.0 mg vitamin E kg^{-1}.

The calves were divided into 3 groups, 18 animals in each (9 bulls and 9 heifers). In our statistical analyses, all the calves were treated in the same manner, without making a distinction between the genders. The control group (CTR) consisted of calves after the colostral period which were watered milk replacer formulation without any supplementation The second (TRT1) and the third (TRT2) group of calves were given milk replacers supplemented with β-carotene (TRT1 group) in the amount of 25 mg/calf/day (10% dry powder, Lucarotin 10% Feed BASF, Ludwigshafen, Germany) or a suspension of liver omega-3 oil (TRT2 group) (EPADMA OIL 4200 Omega 3, TINE B.A. Ingredients, Oslo, Norway, Table 3) in the amount of 5 g/calf/day (Table 2). The scope of the experiment comprised the determination of IgG in the colostrum from mother cows at the first, third and fifth day after calving. This stage was followed by an analysis of selected biochemical and nonspecific

Table 3

Composition and quality of fish oil (omega-3) used in TRT2*

Item, % unless noted	Value
Free Fatty Acids	0.1
SFA	15.2
MUFA	47.5
PUFA	31.4
Total Omega-3 (FA)	27.2
Peroxide values, mEq	0.3
Anisidine value	1.3
Eicosapentaenoic acid (g 100 g^{-1})	8.7
Docosahexaenoic acid (g 100 g^{-1})	13.1

* of 100 g of EPADHA 4200 as reported by the manufacturer (Tine, Oslo, Norway)

humoral immunity indicators in the serum of calves aged 1, 3, 5, 15, 30 and 60 days, including the indicators of:

– total protein (TP), glucose (GLU), total cholesterol (CHOL), triglycerides (TG), creatinine (CRE), urea (UREA), hepatic enzymes, i.e. alanine aminotransferase (ALT) and aspartate aminotransferase (AST), alkaline phosphatase (ALP) and L-lactate dehydrogenase (LDH-L) activity content;
– level of IgG, lysozyme (LYS), ceruloplasmin (CER).

Additionally, to determine the efficiency of rearing, the calves were weighed on the first day after birth, and then on day 30 and 60 of life, based on which daily weight gains during the whole period were calculated. Cases of diarrhoea or upper respiratory tract diseases among the calves were monitored.

Levels of Ig and TP were measured according to the colorimetric micromethod developed by Lowry et al. (1951) (Sigma, Diagnostic Kits) and modified by Siwicki and Anderson (1993). The activity of LYS was determined by the turbidimetric method of Parry et al. (1965) modified by Siwicki and Anderson (1993), and the activity of CER was evaluated according to Siwicki and Studnicka (1986). The activities (U I^{-1}) of ALT, AST, ALP and LDH-L were measured according to the IFCC kinetic method. The concentrations (mg dL^{-1}) of CRE, UREA, TG, GLU and CHOL were determined by a modified Jaffe method (CRE), urease – by the glutamate dehydrogenase UV method (UREA), glucose oxidase – by the peroxidase method (GLU), glycerokinase – by the peroxidase method (TG) and cholesterol – by the oxidase-peroxidase method (CHOL). All biochemical measurements were performed on a MINDRAY BS-120 biochemical analyser (Mindray Medical International Ltd) using diagnostic kits (Alpha Diagnostic). For interpreation of the results, reference levels adopted in the developed standards for cattle were applied (Table 4).

Table 4

Standard activity of the biochemical and immunological indicators submitted to analyis

Parameters	Standard	Source
GLU	81-117 mg dL^{-1}	Andrews et al. (2004), Baumgarnter (2005), Dirksen et al. (2007), Winnicka (2008)
CHOL	70-201 mg dL^{-1}	
TG	8,8-26 mg dL^{-1}	
ALT	25-74 lU	
AST	58-100 lU	
ALP	41-116 lU	
UREA	10-45 mg dL^{-1}	
CRE	1,0-2,1 mg dL^{-1}	
LYS	1-3 mg L^{-1}	Amadori et al. (1997); Knowles et al. (2000)
CER	100-200 IU	Radostis (2007)
TP	51-71 g L^{-1}	Winnicka (2008)
IgG	> 10 g L^{-1}	Amadori et al. (1997)
LDH-L	692-1445 IU	Jackson, Cockcroft (2002)

The IgG immunoglobulin content in colostrum was determined using Bethyl kits (E10-118, E103 and E115). Blood from calves was collected to Vacuette Serum Separation heparinised tubes, left to coagulate at 36°C and subsequently centrifuged (10 min s^{-1} · 3000 rpm, MPW 223e centrifuge). Serum was collected and frozen at -20°C until analyses.

The results were statistically processed according to the univariate analysis in an orthogonal system, supported by Statistica ver. 9.0 (StatSoft 2011). The mean ($\bar{x}$) and standard deviation (SD) were calculated. The significance of differences was verified using the Fisher's LSD test.

RESULTS

The content of immunoglobulin in cows' colostrum and in serum of their calves sampled on the same days during the colostral period is specified in Table 5. On the first day after calving, the IgG content in colostrum was from 82.66 g l^{-1} (among mothers of the CTR group) to 74.03 g l^{-1} (mothers of calves in the TRT2 group). Statistical differences were not confirmed. According to Stefaniak and Jawor (2006), such colostrum would be classified as having good quality. However, the quality of the analysed colostrum declined rather rapidly, which was confirmed by the falling concentration of immunoglobulin. Simultaneously, the efficiency of immunoglobulin absorption through the walls of the small intestine to the circulatory system of calves decreased as well.

Table 5

The level of IgG (g L^{-1}) in the colostrum of cows and in the serum of their calves

Group	Specification	S.M.	Day period of colostrum		
			1	3	5
CTR	colostrum	$\bar{x}$	82.66^{A}	2.28^{A}	1.07^{a}
		SD	4.86	0.79	0.11
	serum of calf	$\bar{x}$	16.16	12.74^{a}	8.23^{a}
		SD	0.73	0.95	0.50
TRT1	colostrum	$\bar{x}$	78.84^{B}	2.13^{A}	1.16^{a}
		SD	2.16	0.73	0.16
	serum of calf	$\bar{x}$	16.10	11.28^{b}	9.74^{b}
		SD	0.77	0.65	0.80
TRT2	colostrum	$\bar{x}$	74.03^{C}	3.04^{B}	0.89^{b}
		SD	2.73	0.88	0.13
	serum of calf	$\bar{x}$	16.80	11.74^{b}	10.09^{c}
		SD	0.91	0.52	0.61

Average between groups: *A*, *B*, *C* $p \leq 0.01$; *a*, *b*, *c* $p \leq 0.05$

The IgG content range in the serum of calves on the first day of colostrum administration varied between groups of calves, slightly exceeding 16 g L^{-1} (Table 5). Our results are in accord with the data reported by Zachwieja et al. (2004). On the third day after calving, the IgG content in colostrum decreased down to the range from 2.13 g L^{-1} to 3.04 g L^{-1}. The IgG level in serum of calves did not show as much of a decrease. In the CTR group of calves, the IgG content was 12.74 g L^{-1}, significantly outperforming ($p \leq 0.05$) the content of IgG in serum of the TRT1 group calves. The lowest content of IgG on the fifth day of the colostral period was found in colostrum administered to calves of the TRT2 group (0.89 g L^{-1}). For the other two groups, the IgG content exceeded 1g L^{-1}. The lowest content of IgG in the serum of all calves from all the groups determined on the fifth day of colostral period appeared in the control group (8.23 g L^{-1}), while being on a similar level (9.74-10.09 g L^{-1}) in colostrum given to calves from the other groups (Table 5).

The level of serum IgG is closely connected with the quality of colostrum and a feeding method. The half-life for antibodies is short, so it is essential to ensure an appropriate level of Ig in serum to avoid FPT (Failure of Passive Transfer). When FPT occurs, calves are more susceptible to infections (Güngör et al. 2004, Hurley, Theil 2011). In our research, no differences between the groups were observed at 1 day. Additionally, a level of Ig higher than >16 g L^{-1} shows that calves received an appropriate amount of colostrum and were fed properly. In our study, both treatment groups had higher IgG concentrations. Previous reports showed a beneficial role of omega-3 (Albers et al. 2002, Calder et al. 2002) and βc (Anderson, Theron 1990) on the immune system. During inflammation, neutrophils produce reactive oxy-

gen species (ROS), whose excessive amounts can damage leukocytes, thus inhibiting the efficiency of the immune system's response. Strong antioxidants, such as βc, can protect the immune system and signalling pathways (Bendich 1989). It is assumed that PUFA supports cytokine-mediated cell signalling as well as the synthesis of prostaglandins, interferon and tumor necrosis factor. Similarly, a positive effect of omega-3 fatty acid treatment on calves has been described in several studies (Hill et al. 2007, 2009, Ballou, DePeters 2008).

Both supplements affected significantly IgG concentrations ($p \leq 0.05$) at day 15 (7.18 g L^{-1} in TRT1 and 6.92 g L^{-1} in TRT2) and 60 (14.95 g L^{-1} in TRT1 and 14.35 g L^{-1} TRT2), when increased means were reported compared with CTR (5.26 g L^{-1} and 12.35 g L^{-1} respectively) – Figure 1. Compared with

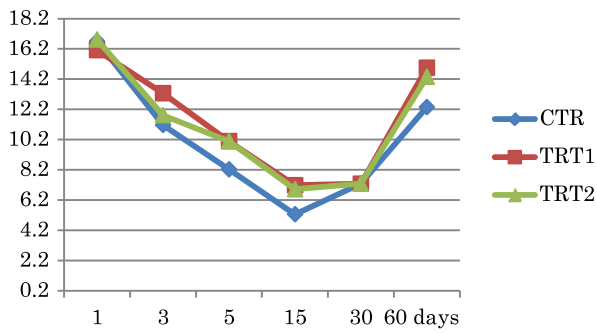

Fig. 1. Serum IgG concentration (g L^{-1})

CTR, both TRT1 and TRT2 had decreased LYS activity ($P \leq 0.05$) at day 15 (0.59 IU vs. 0.70 IU and 0.64 IU) and 30 (0.41 IU vs. 0.62 IU and 0.54 IU) (Figure 2). An addition of βc (TRT1) to a diet increased CER activity ($p \leq 0.05$)

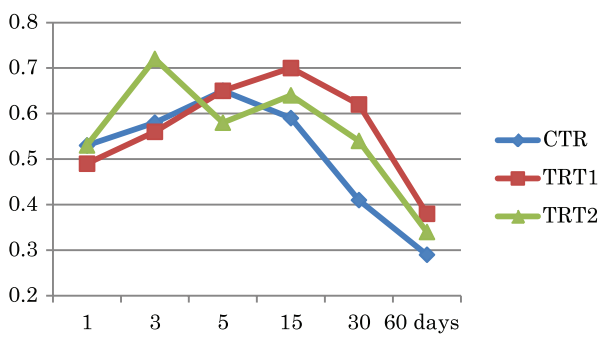

Fig. 2. Lysozyme (LYS) activity (I U^{-1})

in TRT1 and TRT2 compared with CTR, at day 15 (48.11 mg L^{-1}, 48.97 mg L^{-1}, and 53.23 mg L^{-1} respectively). Despite the higher CER activity in TRT2 compared with CTR at day 15 (53.23 mg L^{-1} vs. 48.11 mg L^{-1}) and 30 (47.83 mg L^{-1} vs. 45.52 mg L^{-1}) of life, differences were observed at $p \leq 0.05$ (Figure 3).

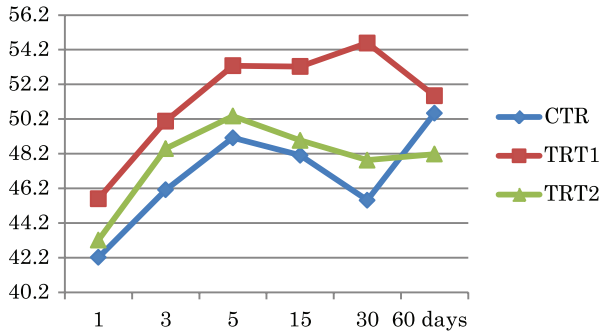

Fig. 3. Ceruloplasmin (CER) activity (mg L^{-1})

In our research, increasing activity of lysozyme was recorded during the colostral period. This can be explained by the intake of colostrum and therefore the ingestion of colostral LYS (Firth et al. 2005, Piantedosi et al. 2010). However, the subsequent decrease can be attributed to either a small amount of blood neutrophils, which are responsible for producing lysozyme, or to the absence of any symptoms of infection (Gueorguiev et al. 1996, Firth et al. 2005). Although the lysozyme activity in this research was lower than reported elsewhere (Biziulevičius et al. 2003, Piantedosi et al. 2010), it must be borne in mind that available data about LYS activity changes in serum of newborn calves are limited.

In our research, an increase of CER activity was observed during the first days of life, which is consistent with the reports by Abeni et al. (2000) and Bertoni et al. (2009). Interestingly, the TRT1 group had higher ADG than CTR, and the TRT2 group presented higher CER activity. It is now widely accepted that mild grade inflammation may occur in obesity (Hotamisligil 2006). However, a higher IgG level and weaker LYS activity in both treatments suggest that the CER level was more probably related to some Cu release from the liver, a phenomenon previously observed in fast growing calves, having a more developed gastrointestinal tract (Abeni et al. 2012). In the absence of clinical signs of illnesses, we can assume that the experimental treatments have a beneficial effect on the liver and its functions, and on the immune system (Chew et al. 1993, Ballou, Depeters 2008).

The effect of *βc* and omega-3 supplementation on *ALT, AST, ALP, LDH-L, GLU, CHOL, TG, TP, UREA* and *CRE* is presented in Figures 4-13. The activity of *ALT, ALP* and *LDH-L* enzymes and blood concentrations of *GLU, CHOL, TG, TP* and *UREA* were affected by the treatment ($p \leq 0.05$). Neither ßc or omega-3 supplements affected the *CRE* level and *AST* activity. All the parameters were affected by the sampling time ($p \leq 0.05$). Calves from TRT1 and TRT2 had decreased ($p \leq 0.05$) *ALT* activity at 15 d compared with CTR (9.0 IU and 9.5 IU vs. 11.7 IU) – Figure 4. Similar observations were made for *AST* (Figure 5), although a significant effect was reported only for the *βc* (TRT1) treatment compared with CTR at day 15 and 30

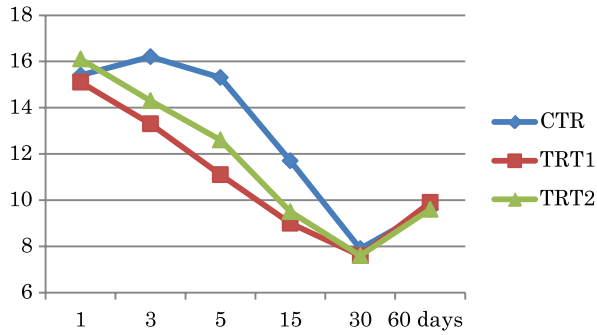

Fig. 4. Alanine aminotransferase (ALT) activity (I U^{-1})

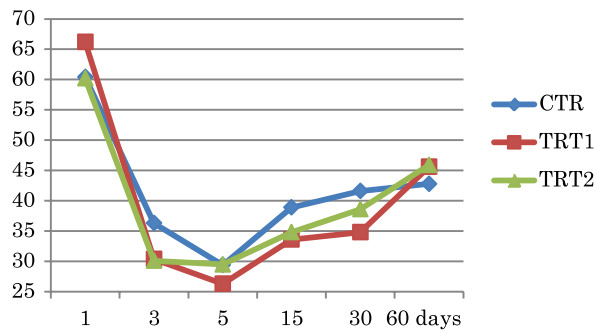

Fig. 5. Aspartate aminotransferase – AST (I U^{-1})

(33.6 IU and 34.8 IU vs 38.9 IU and 41.6 IU respectively). In the TRT1 group, higher *ALP* activity occurred at day 15, 30 and 60 (165.7 IU, 296.2 IU and 325.8 IU) compared with CTR (120.6 IU, 183 IU, and 222.3 IU) and TRT2 (140.9 IU, 190.9 IU, and 272.8 IU). Omega-3 also increased the *ALP* activity, although the difference was statistically significant only at day 60 (Figure 6).

Decreased activity of *ALT* and *AS*T was observed in TRT1 and TRT2 compared with the CTR group. These results are similar to those reported by Mohri et al. (2007), who showed a decreasing tendency for the *AST* activity

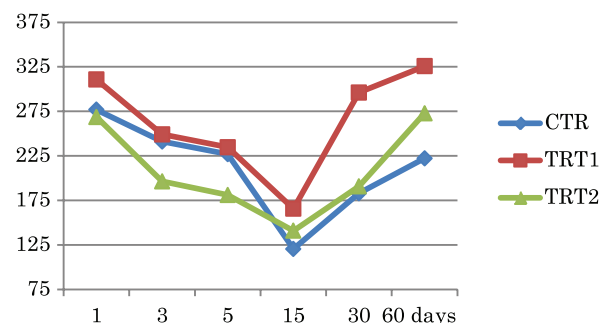

Fig. 6. Alkaline phosphatase (ALP) activity (I U^{-1})

from birth to day 14, and then a small increase at the age of two months. Conversely, several reports (Col, Uslu 2006, Pavlik et al. 2010, El-Deeb, Jacob 2012) indicated an increase in the catalytic *ALT* and *AST* activity during the weaning. Forsyth et al. (1999) suggested that such elevated concentrations of liver enzymes could be used as indicators of the hepatic function. *ALT* and *AST* are enzymes associated with liver parenchymal cells, facilitating the conversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate. Especially *AST* is well known as a nonspecific marker of acute liver damage caused by high-grain diets and associated ruminal lactate production. When body tissue or an organ is diseased or damaged, *AST* is released into the blood; thus, the amount of this enzyme in the blood is related to the extent of damage (Mohri et al. 2007, Castillo et al. 2012, Oler, Głowińska 2013).

The control group (CTR) had decreased means of the *LDH-L* activity ($p \leq 0.05$) at day 15, 30 and 60 (530.9 IU, 735.7 IU, and 834 IU, respectively) compared with TRT1 (627.1 IU, 833.6 IU, and 977.1 IU), and TRT2 (616.1 IU, 882.7 IU, 969.5 IU) – Figure 7. Inclusion of βc (TRT1) and omega-3 (TRT2) into

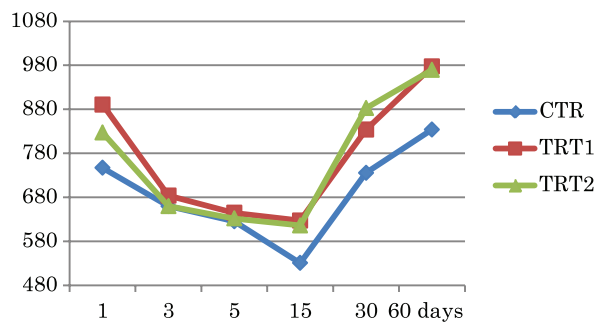

Fig. 7. L-lactate dehydrogenase (LDH-L) activity (IU^{-1})

milk replacer increased *GLU* (108.3 mg dL^{-1} and 93.8 mg dL^{-1} vs. 93.2 mg dL^{-1}), *CHOL* (94.3 mg dL^{-1} and 110 mg dL^{-1} vs. 94.3 mg dL^{-1}), *TG* (28.1 mg dL^{-1} and 29.2 mg dL^{-1} vs. 25.1 mg dL^{-1}) and *TP* (60.9 g L^{-1} and 57.8 g L^{-1} vs. 51.7 g L^{-1}) concentrations ($P \leq 0.05$) compared with CTR (in 60 days) – Figures 8-11. The treatments affected *UREA* concentrations (Figure 12). Calves fed with omega-3 (TRT2) had increased concentrations of this parameter at 30 d and 60 d compared with CTR (20.6 mg dL^{-1} and 17.8 mg dL^{-1} vs. 13.1 mg dL^{-1} and 13.5 mg dL^{-1}, respectively). Calves from TRT1 had a more stable *UREA* concentration curve throughout the whole experiment compared with the other groups. Means of the concentrations reported for TRT1 at 15 and 30 days (18.8 mg dL^{-1} and 16.1 mg dL^{-1}) were different ($p \leq 0.05$) compared with those measured in CTR (27.8 mg dL^{-1} and 13.1 mg dL^{-1}) and TRT2 (26.8 mg dL^{-1} and 20.6 mg dL^{-1}). Both TRT1 and TRT2 had decreased *CREA* concentrations compared with CTR at day 15 (0.91 mg dL^{-1} and 0.95 mg dL^{-1} vs. 0.99 mg dL^{-1}), but no significant treatment effect was reported (Figure 13).

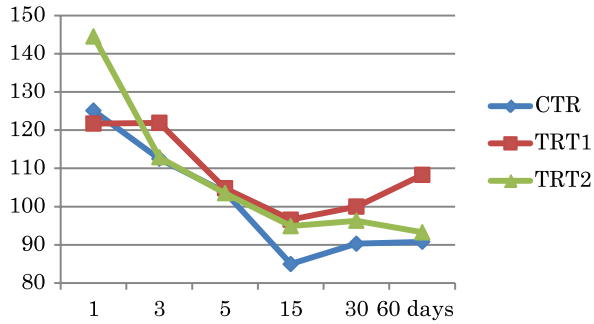

Fig. 8. Glucose (GLU) concentration (mg dL^{-1})

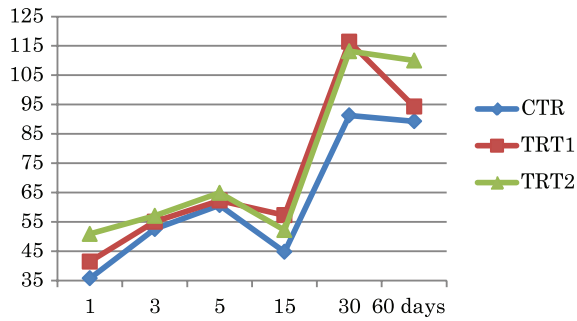

Fig. 9. Cholesterol (CHOL) concentration (mg dL^{-1})

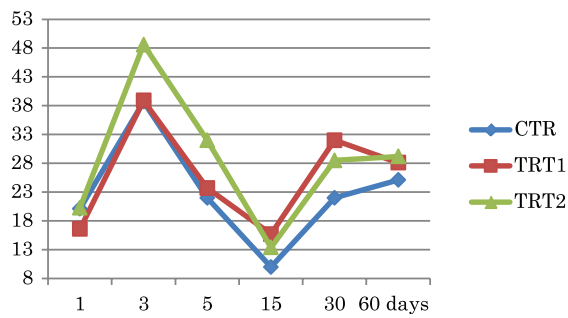

Fig. 10. Triacyloglicerole (TG) concentration (mg dL^{-1})

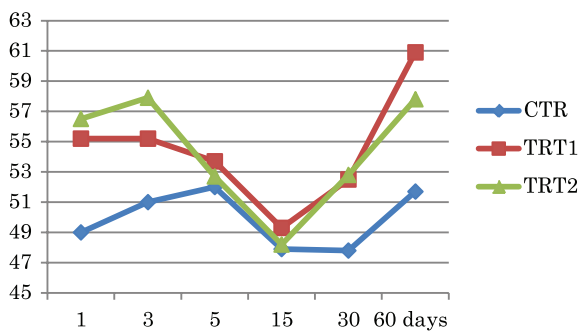

Fig. 11. TP concentration (g dL^{-1})

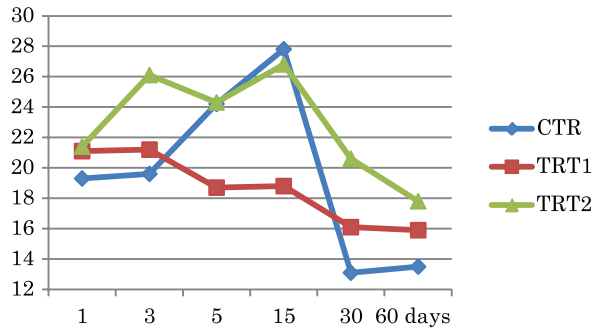

Fig. 12. UREA concentration (mg dL^{-1})

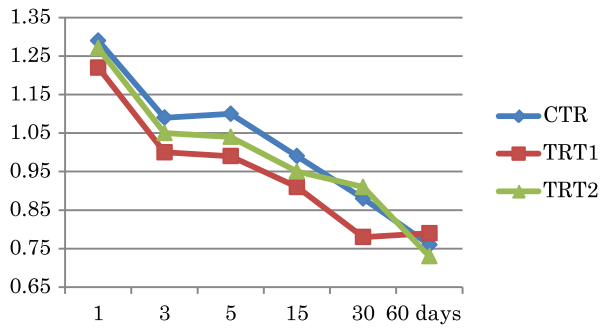

Fig. 13. CREA concentration (mg dL^{-1})

In our research, both treatments affected the *ALP* activity compared with the CTR group. During the first two weeks of life, higher activity of *ALP* can be stimulated by the digestion of colostrum, which is rich in this enzyme (Blum, Hammon 2000, Mohri et al. 2007). Subsequently, the increasing activity is encouraged by the organism's rapid development. During the intensive growth and bone mineralisation, the demand for this enzyme is higher, thus more *ALP* is released from growing bones and into the blood (Knowles et al. 2000, Pavlik et al. 2010). The current results resemble those reported earlier (Knowles et al. 2000, Ndlovu et al. 2009).

The *LDH-L* activity was higher in both TRT1 and TRT2 groups, at all the analyzed points of time, compared with CTR. Moreover, the activity of this enzyme in all the groups was higher at 60 day of life than at birth. A similar tendency was reported in some earlier research on a shorthorn breed of cows (Dornball et al. 1988). Egli, Blum (1998) suggested that the initially higher activity of *LHD-L* could be attributed to the digestion of colostrum, whereas in a later period of life it could be caused by intensive muscular growth. However, *LDH-L* is not specific for muscular tissue, and its activity in the blood serum of ruminants may also increase due to liver disease, skeletal dysfunctions, digestive disorders or pneumonia (Arai et al. 2003, Sobiech et al. 2004, Sobiech, Kuleta 2006). It is worth explaining here that no severe illnesses were observed in our study. Żarczyńska et al. (2012) reported higher

LDH-L activity, but in that case an increase in the *LDH-L* activity in the serum of experimental calves was indicative of dystrophic changes in muscular tissue and was correlated with the observed clinical symptoms.

During the experiment, glucose (*GLU*) concentrations tended to decrease in all the groups. Those finding comply with some previous reports (Cabaraux et al. 2004, Mohri et al. 2007, Pavlik et al. 2010), whereas other researchers (Knowles et al. 2000, Zanker et al. 2001) demonstrated elevated *GLU* levels up to 120 d of life. The serum *GLU* concentration is strictly connected with the feed intake and its quality (Pavlik et al. 2010). Higher *GLU* concentrations in the first period of life can be caused by either colostrum digestion or an after-birth increased corticosteroid level. Later in life, fluctuations in *GLU* concentrations can be attributed to deficient nutrition or intensive muscle growth, which intensifies glycolysis (Bellmann et al. 2004, Khan et al. 2011). Reduced concentrations of *GLU* are attributed to a rise in the concentration of plasma insulin due to an increase in feed intake (Cabaraux et al. 2004).

In our study, *CHOL* and *TG* concentration changes were observed, consisting of an evident decline at 15 day and an increase afterwards. Our results corroborate previous findings (Knowles et al. 2000, Bellmann et al. 2004, Pavlik et al. 2010). Total *CHOL* and *TG* are important indicators of the energy status of young animal. An excessive fat content in a diet increases *TG* accumulation in body, mainly in the liver. This stimulates the acetyl-CoA conversion into ketone bodies, which can induce ketosis or other metabolic disorders (Mu, Porsgaard 2005, Khan et al. 2011). However, the *CHOL* blood serum indicates liver functions, including the proper processing of fatty acids. Decreased *CHOL* concentrations (hypocholesterolemia) cause liver dysfunctions (necrosis, cirrhosis), whereas its higher levels (hypercholesterolemia) increase the level of free fatty acids in blood. In the first period of life, the *CHOL* level in calves may be associated with the digestion of colostrum, which is richer in fat than milk (Pavlik et al. 2010, Khan et al. 2011). Another hypothesis connects a *CHOL* level with serum *GLU* concentrations. Reynolds et al. (2003) suggested that a higher *GLU* level stimulates the secretion of insulin, which reduces the concentration of cyclic adenosine monophosphate (cAMP), thereby stimulating the *CHOL* synthesis (Pavlik et al. 2010). According to several reports, e.g. Cavestany et al. (2005), *CHOL* and *TG* levels may be associated with energy balance. In the present research, fluctuations in the *CHOL* and *TG* levels were similar as reported for *GLU*, and coincided with changes in a diet. This allowed us to assume that the above changes were a feed effect rather than hidden metabolic liver dysfunctions.

Fluctuations in the *TP* level can be used as a diagnostic tool of an organism's hydration (Khan et al. 2011, Oler, Głowińska 2013). Alternatively, measuring the serum *TP* level can be used as a substitute test for estimating the level of passive transfer in calves. In this method, FPT is defined as a serum *TP* concentration of less than 52 g L^{-1} 3 days after birth (Calloway et

al. 2002). In our study, the *TP* level at day 3 was above the required 52 g L^{-1} in all the research groups. Additionally, those results correspond with IgG levels observed in all the groups. A decrease of the *TP* level at day 5 and 15 can be explained by the degradation of Igs received with colostrum. This explanation is suggested in several previous reports (Mohri et al. 2007, Ghorbani et al. 2012).

In our research, the *UREA* level decreased, which agrees with previous reports (Otto et al. 2000, Pavlik et al. 2010). Those changes can be explained by ingested proteins in the feed and their degradation in the rumen, the dietary composition of ingested amino acids, and the capacity for degradation of saccharides in the rumen (Pavlik et al. 2010). However, a significant increase of *UREA* in CTR and TRT2 at 15 d may indicate the impact of diarrhoea on the kidneys, as no increase of CRE was observed in any group (Pekcan et al. 2012). An increased blood *CRE* level may be indicative of renal failure, caused by diarrhoea leading to dehydration, or of a period of insufficient nutrition in animals, when skeletal muscles become a source of energy. Conversely, a decreased level of *UREA*, especially in the first weeks of life, is connected with intensive muscle growth and development (Mohri et al. 2007). According to Adams et al. (1993), serum *CRE* levels are expected to be as high as 0.9-2.5 mg dL^{-1} on delivery but in the first and second day afterwards creatinine levels in calves fall to 0.8-1.9 mg dL^{-1}, thus becoming similar to those in adult animals. This corresponds to our findings as well as some previous reports (Knowles et al. 2000, Mohri et al. 2007).

The monitoring of diarrhoea and upper respiratory tract diseases showed fewer cases in the treatment groups. For example, there were 12% fewer cases of diarrhoea and 18% fewer cases of upper respiratory tract diseases in the TRT1 group than in the control group. Likewise, calves from the TRT2 group suffered less from diarrhoea (16%) and URT infections (10%) than the CTR group.

Birth weight of calves in CTR, TRT1 and TRT2 was 42.67 kg, 43.33 kg and 40.94 kg respectively ($p \leq 0.05$) – Table 6. Calves supplemented with omega-3 (TRT2) had reduced *ADG* (0.502 kg day^{-1}) and *BW* (71.05 kg at 60 days) throughout the whole experimental period compared with CTR (0.523 kg day^{-1} and 74.02 kg at 60 days) and TRT1 (0.584 kg day^{-1} and 78.34 kg at 60 days). However, the difference was statistically verified only in comparison with TRT1 ($p \leq 0.05$). Supplementation with βc increased *ADG* and *BW* compared with CTR but no treatment effect was observed.

Earlier research reported positive influence of fish oil-derived omega-3 FA (Hill et al. 2009, 2011) and βc (Chew et al. 1993) on the health of animals by reducing the number of stool parasites or frequency of diarrhoea while improving the immunological status in mice, calves and humans. Better immunological and biochemical parameters positively affected the growth performance and body development compared with CTR. In the present research, a beneficial role of 30 000 IU kg^{-1} vitamin was observed among the

Table 6

Body weight and average daily gains in calves groups (kg)

Age/periods (days)	Statistical measurements	Groups of calves		
		CTR	TRT1	TRT2
Body weight (BW)				
Birth	$\bar{x}$	42.67	43.33	40.94
	SD	5.24	6.11	589
30	$\bar{x}$	50.87	52.61^{a}	48.22^{b}
	SD	3.87	8.35	5.07
60	$\bar{x}$	74.02	78.34^{a}	71.05^{b}
	SD	6.26	8.66	6.16
Average daily gains (ADG)				
Birth - 30	$\bar{x}$	0.274^{A}	0.309^{B}	0.243^{A}
	SD	0.118	0.10	0.99
Birth - 60	$\bar{x}$	0.523^{a}	0.584^{b}	0.502^{a}
	SD	0.173	0.158	0.110

Average between groups: *A*, *B*, *C* $p \leq 0.01$; *a*, *b*, *c* $p \leq 0.05$

TRT1 calves, which had higher *ADG* and *BW* from birth until the end of observations than calves from CTR and TRT2. Those observations are similar to reported by Nishiyama et al. (2011), although the levels of *ADG* and *BW* detected in our investigation are higher. The lack of a significant difference in *BW* between TRT1 and CTR can be explained by the increased liver weight as a percentage of *BW* (Davis, Rincker et al. 2008). Alternatively, Goncu et al. (2012) reported reduced *ADG* in calves with greater LYS activity, which is consistent with our results. In this study we used fish oil, containing predominantly *EPA* and *DHA*. Those *FA* are considered as the ones affecting neural development and synthesis of particular hormones rather than improving *ADG* (Klein 2002). Previous research by Ballou, De Peters (2008) showed no influence of fish oil supplementation on the growth performance of newborn calves. No effect of *FO* on *ADG* and *BW* has been reported by other authors (Ballou, De Peters 2008, Hill et al. 2009). Our experiment led to consistent observations. The decreased *ADG* and *BW* in TRT2 can be explained by the reduced body fat content in omega-3 supplemented calves. Possible anti-obese properties of *EPA* and *DHA* were suggested by Arai et al. (2009).

CONCLUSIONS

The research showed that the metabolic profile parameters (*ALT, ASP, CHOL, GLU, UREA, ALP, TG, CRE*) were within the range of reference

standards, thus proving a good health status of the calves. Furthermore, the tested supplementation had positive impact on selected immunological parameters, including higher IgG concentration, especially in the late watering period. Beta-carotene supplementation of a diet enabled the TRT1 calves to reach the highest daily gain in the period from 30 to 60 days of life. No such effect was achieved among the TRT2 group of calves, as their body weight was the lowest. However, the beneficial impact of beta-carotene and omega-3 on the non-specific humoral immunity parameters is noteworthy. The effect consisted in fewer cases of clinical diarrhoea and upper respiratory tract diseases.

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