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Assessment of the welfare of cows of the Rhodopean shorthorn breed using blood biochemical parameters

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Abstract

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The blood chemical parameters of 43 free range cows and heifers from 2 farms reared without additional feeding were tested at the end of the pasture period. The cows were from the local, autochthonous Bulgarian Rhodope Shorthorn cattle breed. It was ascertained that the animals exhibited energy deficit with an attempt to compensate it by metabolizing the fat and protein, and a tendency towards pathological processes development. Deficit was also ascertained with reference to the phosphorus content in the ration. It was found that the animals examined had a hyperproteinemia (average values in the farms 78.7-95.0 g/L), hyperglobulinemia (average values 46.7-65.6 g/L) at normal albumin values (average values 29.4-31.9 g/L). The TG (0.414 g/L) LDH (1011.7 U/L), CK (628.1 U/L), ALT (51.86 U/L), GGT U/L (in 32.4% of the animals), AST U/L(in 16.2% of the animals), ALP U/L (in 10.8% of the animals) values were elevated in the farm which displayed more significant deviations of the globulin and the total protein. Considering the characteristics of the region, we can attribute the reasons for the abnormal values to one or a combination of factors connected to: dehydration caused by the decreased intake or excessive excretion of fluids, plant intoxication due to the possible consumption of poisonous plants as a result of poor grazing, fasciolosis infestation in most cases. The authors recommend that in case of free-range breeding of animals without permanent human intervention, the systematic reasons leading to animal discomfort such as underfeeding, thirst, infestation, plant intoxication et al be differentiated and the animals- placed under constant monitoring. The purpose of the above is an early farmer and controlling organizations alert for the arising distress so as timely prevention measures to be taken.

Keywords: animal welfare; comfort; autochthonous breed; hematological parameters; pasture breeding

Introduction

The Rhodope Shorthorn cattle breed is an ancient Bulgarian autochthonous mountain breed (Hinkovski et al., 1984), and basing on a DNA analysis, Hristov et al. (2018) consider that it is the most ancient cattle breed in Europe. The animals are small- the height at the withers of the cows is 104.2 cm (Nikolov, 2012). When compared to the size, the milk yield of the cows is relatively high- 1332 kg, with fat content of the milk of 4.6% (Gadzhev & Nikolov, 2017). Due to this reason, the breed has been used for meeting the milk and dairy products demand in the mountainous regions, mostly in Rila-Rhodope massif, for centuries. The cows are reared in tied stalls alone or in groups of several and are taken out the mountain pastures. Due to their low productivity, the industrial farming of the animals is ineffective and the breed lost its economic significance in the middle of the previous century. Currently it is kept as a national genetic resource. The animals are reared in large herds (over 20 cows) on pasture almost throughout the whole year. The cows are not milked but are used as suckler cows. This has been a sharp change over a short period in both the direction of use and the manner of rearing which, according to Nikolov & Nikolov (2021), is too alarming.

The animal welfare is considered a key element of the modern animal husbandry (Temple & Manteca, 2020) and the pasture rearing- a more favourable, effective and humane manner of cattle rearing (Arnott et al., 2017; Smid et al., 2020). The pastures influence the health of the animals through a set of factors such as botanical composition and climatic conditions directly related to the nutrient balance, the oxidative stress and the behavior of the animals (Nakajima & Yayota, 2019). The pasture farming is also more favourable from environmental point of view in connection with the high-profile heated debates regarding the methane release because this manner of rearing leads to the emission of smaller methane quantities when compared with the quantities released upon feeding with the traditional dairy cow rations (O'Neil et al., 2011). At the same time, a range of analyses emphasise the problems associated with the pasture rearing including discomfort, underfeeding, concerns about chronic hunger (Petherick, 2005; Kondo, 2011; Temple & Manteca, 2020). Thus, Lee at al. (2013) have ascertained that when provided with free access, the cattle actually prefer pastures, spending 2/3 of the day there, however, during active periods of feeding, they go to the additional feeding spots where they meet most of their nutritional needs.

The Eastern- Rhodope pastures where 78.8% of the controlled Rhodope Shorthorn cattle breed is concentrated (Nikolov & Nikolov, 2021) are too poor. The grassland starts developing in March but during most of the years, it starts drying as soon as July or the beginning of August when there is no additional feeding practice during this period.

The blood biochemical parameters are indicative of the physiological and health condition of the animals (Zaitsev et al., 2020) and are used for assessment of the adaptive capacity (Kazhgaliyev et al., 2016) and the welfare of the cattle under different technologies and manners of rearing (Radkowska & Herbut, 2014; Loi et al., 2021).

The study is part of the National Scientific Programe 'Intelligent Animal Husbandry' and the particular aim of the present work is to evaluate the physiological status of cows from the Rhodope Shorthorn cattle breed at the end of the pasture period based on the blood biochemical parameters.

Materials and Methods

The study was carried out in 2021 at the end of the vegetation period (the middle of November- the beginning of December). The blood samples were taken from 43 female animals aged from 18 months to 6 years, reared in two herds- №1- in the village of Obichnik (FA1), Momchilgrad municipality (n = 35), and №2 – in the village of Rakitna, Simitli municipality (n = 8) (FA2). The village of Obichnik is located in the Eastern Rhodopes at an altitude of 356 m. The terrain is slightly uneven. The animals use natural pasture throughout the entire year and are kept indoors only upon snow cover which habitually remains for several days. The village of Rakitna is located in Pirin mountain at an altitude of 776 m. The pastures terrain is highly rocky. The snow cover is retained for longer periods and the grazing period is shorter. The breeding technology in both farms is identical- the animals are reared free in groups with a maximum pasture use. During the winter they are fed with mostly meadow hay and straw.

The blood samples were taken from the caudal vein with the use of lithium heparin vacuum test tubes. The blood parameters were examined within 24 hours after the samples were obtained with an automatic biochemical analyser Seamaty SMT- 120V. The reagent disks used during the analysis cover major biochemical hematological parameters – Glucose (GLU), Total Protein (TP), Albumin (ALB), Total Bilirubin (TB), Creatinine (Crea), Urea Nitrogen (BUN), Triglycerides (TG), Total Cholesterol (TC), Total Bile Acids (TBA), Total Carbon Dioxide/Bicarbonate (tCO_2), Calcium (Ca), Phosphorus (P), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Amylase (AMY), Gamma-glutamyltrasferase (GGT), Lipase (LPS), Lactate Dehydrogenase LDH, Creatine Kinase (CK). The Globulin (GLOB = TP-ALB) and Albumin/Globulin (AG) ratio are automatically calculated.

The reference values for 'cows' set in the software of the biochemical analyser (RBA) are considered as reference ones in our study.

The data were statistically processed variationally with a specialized software (SPSS 21, IBM). The following statistical model was used: $Yijk = \mu + Fi + Aj + FAij + eijk$, where F and A are fixed effects of respectively the i-th farm (n = 2), and the j-th age (n = 2-18-24 months, above 24 months), FA- is a random effect of the age group in the farm, eijk- residual variance.

Results

The bloodchemical parameters examined by us exhibited results which were rather ambiguous and at the same time specific for both farms (Table 1 and Table 2).

There were reliable differences between the farms in most of the parameters examined- TP, ALB, GLOB, Crea, BUN, Ca, the TP/ALB, ALB/GLOB, BUN/Crea ratios, the ALT and AMY enzymes (Table 3). The age group did not have a significant influence on none of the parameters (Table 3) and will not be subject to analysis.

Table 1. Biochemical blood profile of cows and heifers of the Rhodope Shorthorn cattle breed at the end of the pasture period

Parameter	Farm	Mean	±SE	SD	Min.	Max.	Reference values	
							BA[a]	MSD[b]
GLU, mmol/L	1	2.062	0.082	0.484	0.87	3.02	2.0-5.6	2,2-5,6
	2	2.399	0.130	0.367	1.83	2.93		
TP, g/L	1	94.99	1.084	6.414	86.5	114.3	60-75	67-75
	2	78.69	1.221	3.453	71.4	83.2		
ALB, g/L	1	29.44	0.354	2.095	24.7	33.6	25-43	25-38
	2	31.95	0.553	1.565	29.5	34.7		
GLOB, g/L	1	65.55	1.130	6.687	55.3	86.9	30-35	30-35
	2	46.73	1.148	3.246	40.3	50.2		
ALB/GLOB	1	0.451[c]	0.011	0.064	0.32	0.57		
	2	0.688	0.021	0.061	0.59	0.77		
TB, umol/L	1	5.431	0.517	3.060	1.00	15.1	0-27	0-27.4
	2	5.813	0.835	2.361	3.40	10.5		
Crea, umol/L	1	76.46	3.111	18.41	33.8	110.0	44-194	
	2	106.2	5.947	16.82	83.7	130.6		
BUN, mmol/L	1	4.101	0.211	1.245	0.40	8.18	3.6-8.9	
	2	1.601	0.110	0.310	1.28	2.04		
BUN/Crea	1	58.89	3.975	23.18	34.1	133.3	19-202	
	2	15.22	0.992	2.806	11.7	18.69		
tCO_2, mmol/L	1	27.54	0.412	2.439	22.5	32.5	21-27	20-30
	2	28.80	0.953	2.696	24.2	32.2		
Ca, mmol/L	1	2.430	0.020	0.118	2.22	2.68	2.0-2.85	2-2.8
	2	2.333	0.032	0.089	2.16	2.44		
Phos, mmol/L	1	2.064	0.055	0.326	1.18	2.71	1.8-3.3	1.8-2.6
	2	2.130	0.128	0.361	1.61	2.68		
TG, mmol/L	1	0.414	0.024	0.143	0.30	0.86	0.2-0.16	
TC, mmol/L	1	4.453	0.124	0.733	2.97	6.21	1.6-5	
TBA, umol/L	1	29.39	2.469	14.61	9.22	68.02		

**Note:* BA – Biochemical Analyser; **MSD (*MERCK & CO., INC., KENILWORTH, NJ, USA*) MANUALVeterinary Manual; [c] the differences in the parameters are within the statistical error

Low blood glucose levels, elevated total protein and globulin and normal albumin values were observed in animals in both farms. More significant are the deviations of the indicated parameters in the farm in Obichnik (№1). At the moment of blood sampling, the pastures used by the animals in it had a considerably poorer vegetation in comparison with those of the farm in the village of Rakitna.

Formally, the average glucose values were within the reference values set in the biochemical analyser (RBA), however, they were lower than the referential ones specified by MSD and Radostits et al. (2000)- 45 mg/dL (2.5 mmol/L).

Considerable differences both between the two farms (Table 2) and in relation to the reference values were also observed regarding the blood protein content (Table 1). The average value of the total protein levels of the cows from the farm in the village of Rakitna was 4.92% higher than the upper reference value – 75 g/L (RBA) but it was close to it. The value was within the reference values indicated by Alberghina et al (2011) –

Table 2. Some enzymes blood content in cows and heifers of the Rhodope Shorthorn cattle breed at the end of the pasture period

Parameter	Farm №	Mean	±SE	SD	Min	Max	Reference values (RBA)
ALP, U/L	1	119.1	34.61	204.7	30	1176	18-153
	2	50.00	11.18	31.61	24	103	
ALT, U/L	1	51.86	2.716	16.07	31	103	5-35
	2	35.50	3.381	9.561	23	54	
AMY, U/L	1	38.31	1.849	10.93	10	66	41-98
	2	62.38	5.116	14.47	47	86	
AST, U/L	1	105.8	6.274	55.00	37.1	290	60-125*
GGT, U/L	1	17.14	0.707	4.181	11	28	6-17
LPS, U/L	1	22.11	0.268	1.586	19	26	13-60
LDH, U/L	1	1011.7	16.06	94.99	870	1200	309-938
CK, U/L	1	628.1	171.1	1012	54.0	4000	0-350*

**BA – Biochemical Analyser*

Table 3. Influence of the farm and the age category (cows and heifers) on the blood content of some biochemical parameters in Rhodope Shorthorn cattle

Models	Model 1		Model 2
Parameters	Farm	Category	Category*-Farm
TP, g/L	44.15***	1.508	0.149
ALB, g/L	11.43**	0.216	0.932
GLOB, g/L	56.51***	1.068	0.156
Alb/Glob	89.93***	0.047	0.256
TB, umol/L	0.251	1.985	0.138
Crea, umol/L	16.78***	0.043	0.538
BUN, mmol/L	42.05***	1.387	0.142
BUN/Crea	25.64***	1.523	0.147
tCO_2, mmol/L	2.280	3.293	0.330
GLU, mmol/L	2.881	1.274	0.263
Ca, mmol/L	4.627*	0.344	0.549
P, mmol/L	0.059	1.529	0.165
ALT, U/L	6.179*	1.235	0.206
ALP, U/L	2.068	3.232	0.103
AMY, U/L	26.69***	0.002	0.648

***p < 0.001; ** p < 0.01; * p < 0.05

56-79 g/L and it may be considered that it was within the norm when compared with the levels ascertained by other authors for different breeds and technologies of breeding (Kazhgaliyev et al., 2016; Bobbo et al., 2017; Mironova et al., 2021).

The average total protein levels in FA1 were 20.7% higher than those in FA2 (P < 0.001) and were 26.7% above the upper reference value (RBA). Other studies of ours of the breed (Malinova, 2016) carried out in farms in the region during the spring and the autumn ascertained that the total blood protein levels were lower than the reference values- 56.5 and 45.0 g/L respectively. During the present study, in 8 of the animals (22.9% of the tested ones) the total protein values were from 86.5 to 89.6 g/L, in 23 (65.7%) – from 90.7 to 99.4 g/L, and in 4 (11.4%) – from 105.2 to 114.3 g/L.

The increased total protein levels in both farms are associated with the high globulin blood concentration as the albumin levels were within the reference values in all animals. The globulin levels in FA2 were 33.5% above the upper limit, and in FA1, they were twice the normal range. Due to the fact that the albumin levels were higher in the farm with the lower total protein levels (P < 0.01), the ALB/GLOB ratio in FA1 was 34.4% lower than that in FA2 (P < 0.001).

We made additional tests with new parameters in relation to the more significant deviations observed in the parameters examined in FA1. The tests indicated that the triglycerides in the blood were elevated by 2.6 times. The results were also considerably above those ascertained by Kulka et al. (2016) in three cattle breeds (0.07-0.10 mmol/L at the lactation peak, 0.18-0.23 mmol/L during the dry period). The average cholesterol levels of the animals examined by us were within the range reported by the authors indicated and close to the upper reference values (RBA), however, in 21.7% of the animals they exceeded them from 0.4 to 24.2%.

The bile acids in the cattle blood are dynamic and vary widely (Craig et al., 1992). They might be cytotoxic and display pathological effects upon accumulating in large quantities (Blaschka et al., 2020), however, in this particular case, considerable excesses above those reported by the other authors (Craig et al., 1992; Rehage et al., 1999) were not observed.

The total bilirubin also varied in wide ranges with its blood concentration in the cows examined being relatively low, and the difference between the farms- insignificant.

Our previous studies in Rhodopean Shorthorn Cattle have ascertained considerably elevated creatinine levels in the blood (Nikolov et al., 2012; Malinova, 2016). The creatinine concentration during the present study was within the reference values in both farms but the differences between them were significant and reliable ($P < 0.001$). The creatinine levels in FA2 were 38.9% higher than those in FA1. At the same time, the BUN levels (mmol/L) in FA2 were 2.56 times lower ($P < 0.001$) which leads to 3.87 times lower BUN/Crea ratio ($P < 0.001$). The BUN levels in all tested animals from FA2 were lower than the reference values, and the average content was 2.25 times below them. The levels of 29.7% of the animals in FA1 were lower than the bottom reference values.

The average Bicarbonate (mmol/L), Ca (mmol/L) and P (mmol/L) blood levels were within the norm. The calcium level was reliably higher in FA1 ($P < 0.05$) but hypocalcaemia was not observed in any of the animals in both of the farms. The differences between the two farms with reference to the phosphorus levels were insignificant but the individual variation was considerable – from CV – 15.8% in the first one to CV – 16.9% in the second farm. In both farms there were animals with phosphorus levels below the reference values – in 20% of the animals in the first and in 12.5% of the animals in the second farm.

Of all blood enzymes examined in the cattle, only the average ALP (U/L) values were within the reference values, but the variation in FA1 was in very wide ranges (CV-71.9%), and in 4 animals, the levels were from 1.4 to 7.7 times above the norm.

The farm was a reliable source of variation ($P < 0.05$) of the ALT (U/L) blood content of the animals with the enzyme levels in FA1 being 46.1% higher than those in FA2 and 48.2% above the upper limit. The individual variation was lower (CV – 31%) when compared to that of ALP (U/L), but the values over the maximum were registered in almost all animals (92%), and the excess was from 5.7 to 278.4%. Exceeding of over 1.5 times was observed in one third (29.7%) of the animals.

The differences between the farms in terms of AMY (U/L) levels were even more significant ($P < 0.001$). In FA2, the blood levels of the enzyme were close to the intermediate reference values and were within the norm with reference to all animals. The average levels in FA1 were 38.5% lower than those in FA2 and were below the minimum reference values. Lower than the reference values, from 2.4 to 74.6%, were registered in 54.0% of the animals.

Of the enzymes examined in FA1 only, the average levels of LPS (U/L) and AST (U/L) were within the reference values, and GGT (U/L) was slightly above the upper ones. Considering the normal average values of AST, the individual variation was high (CV – 52.0%) and there were values above the upper ones – from 0.8 to 232%, registered in 16.2% of the animals. The variation in GGT was twice as low (CV = 24.4%) but values above the upper ones were registered in 32.4% of the animals with the exceeding being from 5.9 to 64.7%.

The most significant deviations from the reference values were observed with reference to LDH (U/L) and CK (U/L). The average LDH value was 7.86% above the upper reference values. The values of 75.7% of the animals were above the respective limit, and the exceeding was from 12.8 to 27.9%.

Of all enzymes examined, the CK (CV = 61.1%) displayed the most significant variation; the difference between separate individuals was more than 74 times. The values were within the normal range in 73.0% of the animals, and the exceeding in the rest of them was from 8.0 to 142.9%.

Discussion

It is believed the first steps towards ‘humanizing’ the human behavior towards the domestic animals were made more than 200 years ago (Buller, 2009) but according to the author there has been no consensus regarding its definition, content and manner of determination. One thing is clear – the concept has no alternative, and the ‘five freedoms’ (FAWC, 1797) laid in its modern foundations in 1965 have timeless relevance. The first of those freedoms is ‘Freedom from thirst, hunger and underfeeding’.

The glucose is a major source of energy for all higher organisms (Aschenbach, 2010; Mair et al., 2016) and must have persistently adequate levels in the blood so as the metabolic functions to be performed (Abbas et al., 2020). It is directly absorbed by the digestive tract in isomeric form or is a product of the glycogenesis process – a major method for the ruminants (Reynolds, 2005; Aschenbach, 2010).

The low blood glucose levels of the cattle are probabaly typical for the primitive husbandry (Al-Fartosi et al., 2010) but they are also a sign of underfeeding and do not correspond to the concept of humane animal treatment (Mellor, 2016). Glucose levels below 40 mg/dL (2.2 mmol/L) (Mair et al., 2016) and even below 55 mg/dL (3.0 mmol/L) (Megahed, 2018) are considered hypoglycaemic for the dairy cows. 42.9% of the animals in FA1 had glucose levels below 2.0 mmol/L, and only 11.4% had levels above 2.5 mmol/L. Blood glucose levels of below 2.5 mmol/L were registered in 50% of the animals in FA2.

The glucose shortage coming from the feeds may be compensated through glycogenesis (Xu et al., 2020), during the body fats and body protein metabolism, but the breakdown of the latter has a lower activity (Van der Drift, 2012). The glycogenesis is a mechanism for the maintenance of the energy balance upon continuous starvation in which the hepatocytes synthesize glucose, using lactate, pyruvate, glycerol and aminoacids obtained during lipolysis and the muscle glycogen and proteins breakdown (Rui, 2014; Gross et al., 2013).

Probably, the triglycerides increase observed was a result of the attempt to maintain the energy balance through body fat breakdown. Thus, after a three-day food deprivation, people with normal weight increase their triglycerides levels from 28 to 162% (Fainaru & Schafer, 2000). The mechanism of the body reserve breakdown for securing the energy balance is also well- studied in dairy cows at the beginning of their lactation (Bigner et al., 1996; Banos et al., 2005). At the same time, (Chirivi et al., 2022) consider that the intensive, continuous mobilization of the fat increases the risk of metabolic and inflammatory perinatal diseases in dairy cows, and the risk is even higher in cases of endotexemia.

The higher creatinine kinase levels in FA1 ascertained by us are also an indicator of metabolic disorders. The enzyme catalyses the reversible reaction – phosphocreatine (PCr2-)+MgADP+H+ > CK < MgATP2-+ Cr (Wallimann, et al., 2011) and, according to the authors, the CK/PCr system is connected to the energy by means of three physiological principles- it functions as a temporal energy buffer which is immediately accessible; it is a system for energy transportation (energy shuttle or CK/PCr chain); it is a metabolic regulator.

Daroit & Brandelli (2008) point that the CK is a key mechanism of the energy metabolism of tissues with high or unstable needs in vivo such as the skeletal muscle tissue. The authors indicate that the increase of the serum or plasma CK is most commonly used as a skeletal muscles disorder marker. Wang et al. (2020) state that during an experimental starvation in people, especially upon carbohydrate restriction, there is an increase in the blood concentration of uric acid, ALT, CK. As we have already remarked above, the levels of the two enzymes in the animals studied by us were highly elevated.

The above stated shows that at the end of the grazing period, the free-range cows of the Rhodope Shorthorn cattle breed which are not additionaly fed experience extreme discomfort in terms of energy balance. Obviously, the animals are starving which leads to a considerable, pathological, use of bodily reserves for meeting the energy demand. This is too negative due to the fact that considerable reserves are necessary for the maintenance of optimal temperature status during the approaching winter period and the significant deterioration of the climatic conditions. In our previous studies of the breed (Nikolov et al., 2012) we ascertained that during the winter period, the glucose leves were similar to those in FA2 (2.40 mmol/L) but in most of the animals (38.2%), the levels were once again below 2.2 mmol/L.

The phosphorus deficit in the ration must be added to the energy deficiency as it leads the low levels of the element ascertained in the blood of part of the animals. Tolentino et al. (2021) point that the climatic characteristics of the georgraphical region, the breed and the pastures with poor nutritional composition influence the mineral matter in the blood of the cattle. Under conditions similar to ours, Kardaya et al. (2020) have reported close results regarding the presence of minerals in the blood, and Alalade et al. (2021) have registered considerable differences ($P < 0.05$) in the content of Ca, P, K and Mg in the blood of animals grazing on different pastures.

The reasons for the hyperproteinemia in both farms may have different nature. The hyperproteinemia is linked to organism dehydration (Mircheva, 2006; Jennifer & Heller, 2020) which may be due to a reduced fluid intake, excessive fluid loss or both (Jennifer & Heller, 2020). Taking the characteristics of the region where both farms are located into account, both situations can be considered possible. Among the reasons for hyperproteinemia listed by Jennifer & Heller (2020), the possibility if intaking poisonous plants due to the scarce grazing at the end of the pasture period is of particular interest. A similar hypothesis was also supported by the extremely high LDH levels which may be caused by toxicosis caused by plants containing alkaloids and alpha toxins (Klein et al., 2020). Some of the alkaloid-containing plants which are listed by Cortinovis & Caloni (2015) as poisonous for cattle and horses are spread on our territories. These are *Colchicum autumnale, Conium maculatum* and *Datura stramonium*. A *Conium maculatum* poisoning for example was reported in calves in Bulgaria (Binev et al., 2007).

It is clear from Table 1 that the hyperproteinemia was exclusively at the expense of the increased globulin content as the albumin was within the norm. The albumin is a major serum protein (Eckersall, 2008) with a range of physiological functions and, according to Levitt & Levitt (2016), the large number of compounds ensuring most of the plasma antioxidant activity (> 50% in humans) directly connects the albumin serum concentration to the health.

The normal albumin levels indicate that in both farms there was a balance present between the nitrogen intaken with the food and the endogenous needs (Macrae, 2017) at the end of the grazing period. This provides grounds to consider that the animals did not experience protein discomfort which might be observed upon pasture breeding (Nakajima &Yayota, 2019) and which we have observed during our previous studies of the Rhodope Shorthorn Cattle breed during the autumn period (Malinova, 2016), however, according to Friedman & Fadem (2010), the serum albumin is a non-reliable nutritional status marker.

The high globulin levels may stem from different reasons- dehydration, infestation and some chronic inflamations. Judging by the high globulin levels along with the increased liver enzimes levels, fascioliasis infestation may be listed as probable causes for the high globulin values. Phiri et al. (2006) report a strong immune response in the cattle and sheep infestated with *F. hepatica* and *F. gigantica* with the total Ig levels elevating from 3 to 4 weeks after the infection. Chorfi et al. (2004) have ascertained that the total globulin serum concentration correlates strongly with the γ-globulin fractions (r^2 = 0.87) and IgG concentration (r^2 = 0.91). The immunoglobulin levels increase in both natural and artificial contagion (Mezo et al., 2010; Walsh et al., 2021) with *F. hepatica* and the lymphocytic response depends on the severity of the infection (Clery et al., 1996). In a review article, Cwiklinski et al. (2016) summarize that the immune response of ruminants which are naturally infected with *F. hepatica* is well-studied. During the acute phase of the infection, the cattle display a mixed immune response with elevated IL-10, TGF-β, IL-4 and IFN-γ. The Th2/Treg immune responses are those which become dominant as the infection progresses.

Gattani et al. (2018) report that upon fascioliasis infection in cattle the levels of AST, ALT, GGT ALP are increased and there is hypoproteinemia, hypoalbuminemia and hypoglycemia. Increased levels of GGT, which is considered to be an indicator for bile ducts epithelium disorder, are observed in sheep infected with *F. hepatica* (Raadsma et al., 2007). The cattle infested with fascioliasis exhibit: increased levels of AST (Lotfollahzadeh et al., 2008; Nasreldin & Zaki, 2020), ALT (Nasreldin & Zaki, 2020; Brahmbhatt et al., 2021), GGT (Lotfollahzadeh et al., 2008; Brahmbhatt et al., 2021); ALP (Lotfollahzadeh et al., 2008), accompanied by hypoproteinemia, hypoalbuminemia (El-Aziem Hashem & Mohamed, 2017; Brahmbhatt et al., 2021; Frejuk & Stybel, 2021), hypoglycemia (El-Aziem Hashem & Mohamed, 2017), considerable increase of the total bilirubin (Brahmbhatt et al., 2021), elevated creatinine levels (Nasreldin & Zaki, 2020; El-Aziem Hashem &Mohamed, 2017), high globulin levels (El-Aziem Hashem & Mohamed, 2017; Frejuk & Stybel, 2021), and decrease in the A/G ratio (Frejuk & Stybel, 2021).

Klein et al. (2020) indicate that the blood enzymes increase is usually associated with their flow into the the damaged cells. According to Lalor et al. (2021) the parasites assimilate liver tissue and cause an extensive parenchymal destruction with intensive hemorrhagic lesions and immunological reactions. The mechanical damage of the liver is due to the migration of the young methyl. The consequences of liver disorders as a result of the migration damage the liver function, which results in changes in the plasma proteins and liver enzymes concentrations.

In our study the most significant was the increase of the ALT which was observed in all animals, followed by GGT- in 32.4% of the animals, AST- in 16.2% and ALP- in 10.8% of the animals.

According to Giannini et al. (2005) some liver diseases may manifest in a mixed biochemical count- usually there are increased AST and ALT levels and slight abnormalities in the ALP and GGT levels. The authors are of the opinion that after all, the liver damage, notwithstanding if it is acute or chronic, leads to increase in the serum concentrations of aminotransferases, which are in high concentration in the liver. According to the authors, the increase of the ALT serum levels, as observed in our study, is more specific for a liver disorder. Mircheva (2006) report that the ALT levels in the blood may increase from 5 to 100 times in the event of liver diseases.

Giannini et al. (2005) point that in around 80% of the patients suffering from ischemic liver damage, the serum bilirubin levels are lower than 34 μmol/L, the LDH may have very high concentrations, and the ALT/LDH ration < 1. In our study, the TBA of the cattle was below the value indicated, the LDH levels were high, and the ALT/LDH ratio was 0.051.

The low BUN and BUN/Cr values reported in FA2 and the high TP, the normal ALB, and the elevated but within the normal range Crea values might once again be associated with liver disorders and pathological changes in the miscles Hosten et al. (1990).

The high LDH levels ascertained in 75.7% of the animals in FA1 may be attributed to a range of reasons such as: hepatocytes disorder connected to lipidosis, bacterial and necrotic hepatitis, chronic hepatitis and cirrhosis, muscle disorders, plant poisoning et al. (Klein et al., 2020). The above stated analysis along with the enzyme elevation indicate that the lack of animal welfare in the farm is most probably due to a combination of factors which might be differentiated in a more detailed study.

Obviously, in free – range rearing of animals without permanent human intervention, it is necessary to differentiate the systematic reasons leading to animal discomfort such as underfeeding, thirst, infestation, plant poisoning and place them under constant monitoring. The modern IT technologies (Buller et al., 2020) might be used for the purpose. The monitoring would ensure early alert of the farmer and the controlling organizations for an arising distress so that timely prevention measures to be taken.

Conclusion

It was ascertained that at the end of the grazing period, cows from the Bulgarian autochthonous Rhodope Shorthorn cattle breed which were reared free – range and without additional feding exhibited an energy deficit with an attempt to compensate it by metabolizing the fat and protein, and a tendency towards pathological processes development. Deficit was also ascertained with reference to the phosphorus content in the ration.

The results of the animals testing displayed hyperproteinemia (average levels in the farms- 78.7-95.0 *g/L*), hyperglobulinemia (average levels of 46.7-65.6 g/L) at normal albumin levels (average values of 29.4-31.9 g/L). The values of TG (0.414 mmol/L), LDH (1011.7 U/L), CK (628.1 U/L), ALT (51.86 U/L), GGT (in 32.4% of the animals), AST (in 16.2% of the animals) and ALP (in 10.8% of the animals) were also increased in the farm displaying more significant deviations in the globulin and the total protein.

Taking the regional characteristics into account, we can attribute the abnormal values to one or a combination of factors connected to: dehydration caused by the decreased intake or excessive excretion of fluids, plant poisoning due to the possibility of consuming poisonous plants as a result of the poor pasture or infestation, most probably with fascioliasis.

In free-range animal rearing without permanent human intervention, it is necessary to differentiate the systematic reasons leading to animal discomfort such as underfeeding, thirst, infestation, plant poisoning et al and place them under constant monitoring. The monitoring would ensure early alert of the farmer and the controlling organizations for an arising distress so that timely prevention measures to be taken.

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