Enhancement of the glucose metabolism and the reverse cholesterol transport by a peroxisome proliferator receptor α (PPARα) agonist included in the fasciolosis treatment in naturally infested sheep

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SUMMARY

The purpose of this study was to investigate the effects of PPARα agonist on metabolic parameters in naturally Fasciola-infected sheep. Twenty sheep with fasciolosis were allotted in 2 equal groups according to the received treatment (triclabendazole/levamisole in the group I and triclabendazole/levamisole + PPARα agonist (2-methyl-2-phenoxy-propionic acid) in the group II) and the haematological and biochemical parameters were assessed before the treatment and 7, 14 and 28 days after and were compared to control values determined from 10 healthy sheep. As expected, fasciolosis has induced an anaemia evidenced by low haematocrit and low haemoglobinemia coupled to eosinophilia and to a moderate lymphopenia, severe liver damage characterised by hepatocyte degeneration (significant increases in serum AST and GDH activities) and bile duct obstruction (significant increases in serum GGT activity and in serum bile acid concentrations) leading to impairment in liver metabolic functions (hypoalbuminemia, marked decreases in serum glucose concentrations and in LDL associated cholesterol concentrations). All the haematological and biochemical alterations were at least partially alleviated by the antihelminthic treatment except the serum triglyceride concentrations which have continued to decrease during the post-treatment period and the total cholesterol and HDL associated cholesterol concentrations which have markedly increased, particularly when sheep were also treated with the PPARα agonist. These results clearly demonstrated the severe metabolic liver impairment occurring during fasciolosis in sheep and the beneficial effects of the PPARα agonist on the correction of the negative energy balance, particularly by promoting the reverse cholesterol transport.

Keywords: Fasciolosis, sheep, haematological analysis, biochemical analysis, liver, glucose, lipid profiles, cholesterol, lipoproteins, PPARα agonist.

Introduction

The liver fluke, Fasciola hepatica (F. hepatica), is a common parasite of ruminants in many countries in the temperate climates and often causes important production losses in infected animals [1, 9, 11, 14, 29, 39]. Natural fasciolosis in sheep develops clinical manifestations of acute, subacute and chronic infection according to numbers of metacercaria ingested in a period of time [1, 4, 11, 14, 39]. Similarly, the pathogeneity and clinical aspect of fasciolosis are also associated with the number of flukes [1, 4, 11, 24, 39, 40]. The disease occurs in two phases: the parenchymal phase during migration of flukes through the liver parenchyma in which they cause traumatic damage to the parenchyma in the form of necrosis, fibrosis and cirrhosis, and the biliary phase which coincides with their residence in the bile ducts and is characterized by hyperplasia of the bile duct epithelium that eventually leads to cholangitis and biliary obstruction [1, 4,
As the liver plays a central role in the physiology of the body, hepatic pathology due to fasciolosis, even when only small liver areas are damaged, results in significant disturbances and/or changes in components of blood, mitochondrial bioenergetic metabolism, carbohydrate, protein, lipid and steroid metabolisms as well as bile flow and composition [4, 11].

Common blood changes occurring in infected hosts are anaemia, eosinophilia, hypoalbuminaemia and increased liver enzyme activities as reported by several researchers [1, 4, 11, 29, 38, 39, 47]. For all that happens, researchers in recent years focused on the metabolic effect on energy deficiency in fasciolosis [14, 27, 36]. In this regard, PHIRI et al. [36] showed that fasciolosis caused energy deficiency (low glucose) and ketosis (increased β-hydroxybutyrate) in experimentally infected sheep. This situation can be explained by the fact that the migrating flukes cause severe liver pathology and death of hepatocytes, which may potentially reduce liver glycogen reservoirs [4, 27, 36] and the consequent reduction in available liver glycogen leads to a reduction in glycemia [15, 36] and a corresponding increased mobilisation of free fatty acids (FFA) [36] and secondary increased serum concentrations of ketone bodies such as β-hydroxybutyrate [4, 22, 36]. However, JEMLI et al. [21] reported that sheep infected with 200 metacercariae exhibited no change in serum triglyceride and cholesterol concentrations over 14 weeks after infection. Likewise, infected sheep with *F. hepatica* showed no change in FFA concentration and phospholipid composition or content in liver mitochondria isolated 4 weeks post-infection [27]. On the other hand, mitochondria isolated from infected livers were reported to contain elevated concentrations of FFA [26]. Moreover, it is suggested that although bile flow and secretion decreased, the serum bile acid concentrations were increased in *F. hepatica* infected sheep which it may be signs of parenchymal degeneration of the liver [14]. In addition, authors reported that the liver antioxidant capacities in the course of fasciolosis were reduced whereas the reactive oxygen species genesis enhanced during the infection [24, 40].

The peroxisome proliferators activated receptor α (PPARα) belongs to the nuclear hormone receptor family [10, 25, 34, 41]. Although PPARα is expressed in all tissues of mammals [10, 25], it is mainly expressed in the tissues which have a highly efficient mitochondrial fatty acid oxidation such as liver, brown adipose tissue, kidneys, skeletal muscle and heart [23, 25, 41, 46]. They are considered as transcription factors activating by lipids and regulate genes controlling lipid and glucose metabolism and adipogenesis. In the liver, PPARα directly regulates genes involved in fatty acid uptake and oxidation and gluconeogenesis. Finally, PPARα stimulates hepatic fatty acid oxidation, ketogenesis and conversion of glycerol to glucose [10, 13, 23, 25, 34]. In brief, it regulates metabolism of lipid and lipoprotein, as well as glucose homeostasis [7, 10, 34, 46]. In accordance with above mentioned subjects, it was previously reported that the application of PPARα agonist reduced the serum concentrations of low density lipoprotein cholesterol (LDLc), triglycerides (TG) and FFA but increased high density lipoprotein cholesterol (HDLC) and glycaemia [7, 18, 23, 30, 34, 46]. In addition, HUBER et al. [20] have shown that the PPARα agonist, nafenopin, reduces lipid peroxidation in rat liver. Moreover, it is shown that the activation of PPARα in rodent liver and in primary hepatocyte cultures promotes hepatocyte proliferation and regeneration and reduced apoptosis [3, 19], and that the liver regeneration in PPARα-null mice is impaired following partial hepatectomy [2, 12]. Furthermore, authors reported that in PPARα-null or deficient mice at laboratory conditions suffer from metabolic defects including hypoglycaemia, hypoketonemia, hypothermia and elevated plasma FFA concentrations [8, 22, 35, 41]. Because the administration of a selective PPARα agonist has been shown effective for the treatment of fatty liver, ketosis, acidosis, digestive disorder, intoxications and impaired hepatic activity [13], and because its specific and broad-scale action on energy metabolism, choleresis and inflammation represents a first line of therapy for the treatment of metabolic disorders, the PPARα agonist usage in veterinary medicine has developed in recent years. Moreover, same authors [13] have shown that the administration of PPARα agonist has also caused increase in choleresis.

If above mentioned knowledge in related to fasciolosis and PPAR α agonist evaluated, the usage in the treatment of fasciolosis may have useful effect on metabolic changes developed in fasciolosis. For this reason, in the present study, the effects of PPARα agonist on metabolic profiles such as glycaemia, bile acid concentrations and lipid profiles in sheep with natural chronic fasciolosis were aimed to investigate.

**Materials and Methods**

**ANIMALS AND STUDY DESIGN**

This study was carried out in Özalp district of Van province (Turkey) between February and April 2008. According to anamnesis and clinical examination ( cachexia, anorexia, mess in wool, oedema under the mandibula, paleness in the mucous membranes), fasciolosis was suspected in 65 Akkaraman sheep from the same flock, 1-3 years old. After the diagnosis confirmation by evidencing *Fasciola* spp eggs in stool samples, 20 *Fasciola*-affected sheep and 10 “healthy” sheep whith no *Fasciola* spp eggs in stool samples were included in the assay. Then, diseased animals were allotted into two equal groups (n = 10) as group I and group II in which egg numbers in the stool samples and packed cell volume (PCV) values were taken into consideration.

All animals (in assay and control groups) orally received 10 mg/kg body weight triclabendazole and 7.5 mg/kg levamisole (Levatrizol™, Vetas'/Turkey). In addition, diseased animals in the group II were intramuscularly treated by a PPARα agonist, the 2-methyl-2-phenoxy-propionic acid (Hepagen™, Vetas'/Turkey), at the dose of 0.1 mg/kg twice with one week interval (on days 0 and 7).
In the assay group, blood samples for haematological and biochemical investigations were collected from jugular vein into sterile tubes with and without anticoagulant, respectively before treatment (BT) and 7, 14, 21 and 28 days after. Blood samples in controls were collected only once. For haematological analysis, whole blood samples were stored at +4°C and analyzed within maximum 4 hours. For biochemical analysis, after clotting for 1 hour at room temperature and centrifugation (Rotofix 32®-Hettich, 4 000g, 10 minutes, room temperature), sera were carefully harvested and stored at -20°C until analysis.

In parallel to blood sampling, faecal samples were collected from the rectum and the number of *F. hepatica* eggs was determined by flotation-centrifugation method according to the modified Mc MASTER technique [43].

**HAEMATOLOGICAL AND BIOCHEMICAL ANALYSES**

The haematological parameters (haemoglobin (Hb) concentrations, Packed cell volume (PCV) values and white blood cell (WBC) counts) were analysed using QBC® Vet Autoread™ cell counter (IDEXX/USA). Differential WBC counts were also determined manually from Giemsa stained smears by classic staining method.

Serum glucose, total protein, albumin, globulin, total bilirubin (TBil), triglyceride, total cholesterol (TC), HDL- and LDL-associated cholesterol (HDLc and LDLc) and total lipid (TLIP) concentrations as well as the aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) activities were analysed using biochemical autoanalyzer (Hitachi 902® -Roche & Boehringer Mannheim). Similarly, serum bile acid concentrations were determined by commercial kits (Sigma Bile Acid Kit 450-SIGMA-ALDRICH®/Germany) and the serum glutamate dehydrogenase (GDH) activity was determined by a commercial kit (Random-GL442/UK) using spectrophotometer (Photometer® 5010, Boehringer-Mannheim /Germany).

**STATISTICAL ANALYSIS**

For haematological and biochemical findings, statistical evaluation between control group and differently treated groups (group I and II) were analysed using independent samples t test and the variations of the parameters according to time were analysed using paired samples t test. The treatment efficacy (or % reduction) for liver fluke faecal egg counts for each treatment group was expressed in percentage after deducting the egg counts measured after treatment from initial egg counts [45] and the statistical evaluation between groups I and II for the same time point were analysed using paired samples t test. For this purpose, the SPSS 12.0 software was used. Statistical significance was set at p < 0.05. All data were expressed as means ± standard error of the mean (S.E.M).

**Results**

The results of faecal examinations according to time are illustrated in figure 1. Before treatment, the mean values of *Fasciola* spp egg counts found in stool samples of diseased animals (groups I and II) were 650.0 ± 64.5 and 541.6 ± 70.7, respectively. After treatments, the faecal *Fasciola* spp egg excretion was dramatically reduced in both 2 groups during the whole 28 days post-treatment period (p < 0.001) compared to the initial values but although the egg counts measured during this period in the faecal samples from sheep treated with triclabendazole/levamisole and the PPARα agonist (group II) were systematically inferior to values obtained in the group I (treated with triclabendazole/levamisole), the differences between the 2 treated groups were not statistically significant whatever the time point considered. In both 2 groups, the treatment efficacy has gradually increased according to time from around 90% on day 7 to around 95% on day 28 and has not significantly differed between the 2 treated groups.

![Figure 1: Time variations of the mean Fasciola spp egg counts in faeces from sheep with chronic fasciolosis treated on day 0 with triclabendazole (10 mg/kg) /levamisole (7.5 mg/kg) orally (group I) or with triclabendazole (10 mg/kg) /levamisole (7.5 mg/kg) orally plus a PPARα agonist (2-methyl-2-phenoxy-propionic acid, 0.1 mg/kg) intramuscularly on days 0 and 7. Results are expressed as means ± standard error of the mean (S.E.M).](image)

***p < 0.001 between initial values (Day 0) and the other time points.

The haematological findings in sheep with chronic fasciolosis are presented in Table I. The haematocrit values and the haemoglobinemia were dramatically depressed in both infected groups before treatment (day 0) compared to the control values (p < 0.01). After administration of triclabendazole and levamisole eventually associated to the PPARα agonist, they gradually increased according to time (day 0 vs. days 7, 14 and 28: p < 0.05, day 7 vs. days 14 and 28: p < 0.05 for haematocrit values; day 0 vs. days 14 and 28: p < 0.05 for haemoglobinemia) but they remained significantly
YÜKSEK (N.) AND COLLABORATORS

Table I: Time variations of the haematological parameters in sheep with chronic fasciolosis treated on day 0 with triclabendazole (10 mg/kg) /levamisole (7.5 mg/kg) orally (group I) or with triclabendazole (10 mg/kg) /levamisole (7.5 mg/kg) orally plus a PPARα agonist (2-methyl-2-phenoxypyropionic acid, 0.1 mg/kg) intramuscularly on days 0 and 7. Results are expressed as means ± standard error of the mean (S.E.M).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (triclabendazole/levamisole)</th>
<th>Group II (triclabendazole/levamisole + PPARα agonist)</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 7</td>
<td>Day 14</td>
</tr>
<tr>
<td>PCV (L/L)</td>
<td>0.24</td>
<td>0.26</td>
<td>0.28</td>
</tr>
<tr>
<td>Hb (g/L)</td>
<td>83.6</td>
<td>88.7</td>
<td>97.2</td>
</tr>
<tr>
<td></td>
<td>± 0.02a</td>
<td>± 0.01b</td>
<td>± 0.01b</td>
</tr>
<tr>
<td>MHCC (g/L)</td>
<td>344.7</td>
<td>341.8</td>
<td>344.1</td>
</tr>
<tr>
<td></td>
<td>± 45.5</td>
<td>± 44.0</td>
<td>± 45.6</td>
</tr>
<tr>
<td>WBC (10⁹/L)</td>
<td>8.13</td>
<td>7.10</td>
<td>7.44</td>
</tr>
<tr>
<td></td>
<td>± 0.52</td>
<td>± 0.65</td>
<td>± 0.43</td>
</tr>
<tr>
<td>Neutrophil (10⁹/L)</td>
<td>3.13</td>
<td>2.95</td>
<td>3.14</td>
</tr>
<tr>
<td></td>
<td>± 0.52</td>
<td>± 0.16</td>
<td>± 0.49</td>
</tr>
<tr>
<td>Eosinophil (10⁹/L)</td>
<td>0.57</td>
<td>0.16</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>± 0.06a</td>
<td>± 0.03a</td>
<td>± 0.03a</td>
</tr>
<tr>
<td>Basophil (10⁹/L)</td>
<td>0.06</td>
<td>0.05</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>± 0.03</td>
<td>± 0.01</td>
<td>± 0.03</td>
</tr>
<tr>
<td>Lymphocyte (10⁹/L)</td>
<td>4.21</td>
<td>3.80</td>
<td>3.91</td>
</tr>
<tr>
<td></td>
<td>± 0.57a</td>
<td>± 0.18b</td>
<td>± 0.46b</td>
</tr>
<tr>
<td>Monocyte (10⁹/L)</td>
<td>0.16</td>
<td>0.14</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>± 0.05</td>
<td>± 0.02</td>
<td>± 0.03</td>
</tr>
</tbody>
</table>

PCV: Packed cell volume; Hb: Haemoglobin concentration; MHCC: Mean Haemoglobin Corpuscular concentration (Hb/PCV); WBC: White Blood Cell count.

Different superscripts a,b,c,d in the same row indicate significant differences (p < 0.05) between groups.

decreased compared to the healthy controls until the 7th day and the 14th day (p < 0.05) for the haemoglobinemia and the haematocrit, respectively. On day 28, these 2 parameters were still lower in both infected groups than in the control group but not significantly. No significant difference between the both infected groups was evidenced. In parallel, the mean haemoglobin corpuscular concentrations (MHCC) remained stable in infected animals.

Although the leukocyte counts appeared slightly reduced before treatment in diseased sheep, the observed time variations were not significant compared to the control values. The neutrophil, basophil and monocyte populations remained relatively stable whereas a moderate lymphopenia (p < 0.05) was noted before treatment (group II) and still persisted on day 7 in sheep only treated with triclabendazole and levamisole (group I) and the eosinophil population was significantly extended compared to controls before treatment (p < 0.01) in both infected groups then gradually declined according to time after treatment.

The biochemical findings are summarized in Table II. Whereas the globulinemia was unchanged, the proteinemia and the albuminemia were significantly lower in Fasciola-infected sheep before treatment than in the healthy controls (p < 0.01 and 0.05 for proteinemia in groups II and I, respectively; p < 0.001 for albuminemia). Thereafter, the 3 parameters progressively increased according to time after treatment (day 0 vs. day 28: p < 0.05). The proteinemia were still significantly depressed compared to the controls 7 days after treatment in the 2 infected groups (p < 0.05 for the group I and p < 0.01 for the group II) with the same intensity (group I vs. group II: not significant) and reached values similar to the controls on day 28, and the albuminemia remained markedly reduced for 28 days (day 7: p < 0.001; days 14 and 28: p < 0.01 in the group I and < 0.001 in the group II), particularly in the group II (treated by triclabendazole/levamisole and the PPARα agonist) (group I vs. group II on day 28: p < 0.05). On the other hand, the globulinemia significantly increased compared to the controls (p < 0.05 for the group I and p < 0.01 for the group II) and the pre-treatment values (p < 0.01) since the 14th day.

The serum bile acid concentrations in both treatment groups dramatically increased before treatment compared to the control group (p < 0.01) and then they slowly declined (day 0 vs. day 28: p < 0.05), particularly in the group II in which they remained significantly increased on days 14 and 28 (p < 0.05). For the total bilirubine concentrations, although this parameter was slightly increased before treatment, no significant difference between infected and healthy sheep was observed during the experiment.

Significantly low glucose concentrations were found in Fasciola-infected sheep before treatment (p < 0.001) but since the 7th day after treatment, they have significantly
increased in both infected groups (day 0 vs. day 7; \( p < 0.05 \)). However, they remained still significantly depressed in the group I (receiving only Triclabendazol and levamisole) on days 7 and 14 (\( p < 0.01 \)) but at these 2 dates, no significant difference was observed between the 2 infected and treated groups (Table II).

Although slightly lowered in infected sheep, the total lipidemia has not significantly changed compared to controls during the whole experimental period. Before treatment, the triglyceride concentrations were weakly but not significantly lowered in infected sheep. After treatment, while the triglyceride concentrations progressively decreased according to time (days 0 and 7 vs. days 14 and 28; \( p < 0.05 \)) and became significantly lower than the control concentrations on days 7 (\( p < 0.05 \)), 14 and 28 (\( p < 0.01 \)), the serum total cholesterol concentrations slowly increased for significantly standing higher than control values on day 28 when sheep have received PPAR\( \alpha \) agonist and triclabendazole and levamisole (group II) (\( p < 0.05 \)). In the same way, the HDL associated concentrations (HDLc), already slightly elevated before treatment compared to the control values, have also gradually increased according to time (day 0 vs. day 28; \( p < 0.05 \)) in infected sheep after treatment and reached significantly higher values than controls on day 28 (\( p < 0.05 \)) for the group I (treated with triclabendazole/levamisole) and since the 7th day for the group II (treated with triclabendazole/levamisole + PPAR\( \alpha \) agonist) (Days 7 and 14: \( p < 0.05 \); day 28: \( p < 0.01 \)). Furthermore, this parameter on day 28 was significantly higher in the group II than in the group I (\( p < 0.05 \)). By contrast, the serum LDL associated concentrations (LDLc) were drastically lowered in both infected groups compared to the control group before treatment (\( p < 0.01 \)) and although they have weakly increased after treatment, they remained still significantly depressed in the group I (receiving only Triclabendazol and levamisole) on days 7 and 14 (\( p < 0.01 \) but at these 2 dates, no significant difference was observed between the 2 infected and treated groups (Table II).

### Table II: Time variations of the biochemical parameters in sheep with chronic fasciolosis treated on day 0 with triclabendazole (10 mg/kg) /levamisole (7.5 mg/kg) orally (group I) or with triclabendazole (10 mg/kg) /levamisole (7.5 mg/kg) orally plus a PPAR\( \alpha \) agonist (2-methyl-2-phenoxy-propionic acid, 0.1 mg/kg) intramuscularly on days 0 and 7. Results are expressed as means ± standard error of the mean (S.E.M).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group II (triclabendazole /levamisole + PPAR( \alpha ) agonist)</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>Day 7</td>
<td>Day 14</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>63.9 ± 4.5</td>
<td>65.2 ± 3.8</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>23.0 ± 2.1</td>
<td>23.2 ± 1.5</td>
</tr>
<tr>
<td>Globulin (g/L)</td>
<td>40.9 ± 2.9</td>
<td>42.0 ± 3.1</td>
</tr>
<tr>
<td>Bile acid (( \mu )mol/L)</td>
<td>80.1 ± 7.9</td>
<td>71.0 ± 14.9</td>
</tr>
<tr>
<td>TBil (mmol/L)</td>
<td>2.89 ± 0.05</td>
<td>2.04 ± 0.03</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>2.47 ± 0.14</td>
<td>2.90 ± 0.19</td>
</tr>
<tr>
<td>Triglycerides (mg/L)</td>
<td>21.72 ± 4.05</td>
<td>17.65 ± 2.54</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>1.40 ± 0.16</td>
<td>1.57 ± 0.22</td>
</tr>
<tr>
<td>HDLc (mg/L)</td>
<td>406.9 ± 50.7</td>
<td>457.0 ± 67.7</td>
</tr>
<tr>
<td>LDLc (mg/L)</td>
<td>93.0 ± 13.6</td>
<td>119.3 ± 21.4</td>
</tr>
<tr>
<td>TLIP (g/L)</td>
<td>3.05 ± 0.15</td>
<td>3.14 ± 0.19</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>124.0 ± 8.3</td>
<td>104.5 ± 5.5</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>15.83 ± 2.26</td>
<td>14.00 ± 2.81</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>90.0 ± 2.00</td>
<td>90.3 ± 15.5</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>80.8 ± 4.5</td>
<td>75.4 ± 3.5</td>
</tr>
<tr>
<td>GDH (U/L)</td>
<td>12.43 ± 4.5</td>
<td>7.51 ± 3.5</td>
</tr>
</tbody>
</table>

TBil: Total Bilirubine; TC: Total cholesterol; HDLc: HDL associated cholesterol; LDLc: LDL associated cholesterol; TLIP: Total lipid; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; GGT: \( \gamma \) Glutamyl transferase; GDH: glutamate dehydrogenase.

Different superscripts \( a,b,c,d \) in the same row indicate significant differences (\( p < 0.05 \)) between groups.

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they were remained still significantly depressed throughout the 28 days post-treatment period (p < 0.01 to p < 0.05) (Table II). On day 28, LDLc concentrations were significantly lower in the group II than in the group I (p < 0.05).

As shown in Table II, the serum AST (p < 0.05), GGT (p < 0.01) and GDH (p < 0.001) activities were dramatically increased in Fasciola-infected sheep compared to the healthy controls before treatment. After treatment, the 3 parameters gradually declined according to time (days 0 and 7 vs. days 14 and 28: p < 0.05 for the GGT and GDH activities; days 0 vs.day 7: p < 0.05 for the GDH activity). However, contrary to the AST activity, the GGT and the GDH activities remained significantly depressed compared to the healthy controls until the 14th day (day 7: p < 0.01, day 14: p < 0.05). On day 28, the enzyme activities, although still low, did not significantly differed from the control values. Furthermore, the variations of the enzyme activities according to time were similar in sheep treated with triclabendazole/levamisole alone or associated to the PPARα agonist. On the other hand, there was no significant difference in the mean ALT and ALP activities before and after treatment between control and the 2 infected groups.

**Discussion**

In earlier studies, several researchers [4, 5, 11, 39, 42] reported that triclabendazole has a high effect against mature and early immature *F. hepatica*. However, development of triclabendazole resistance towards some isolates of *F. hepatica* in sheep in recent years was reported by some authors [16, 44]. In addition, YÜKSEK et al. [47] showed that combination of levamisole and triclabendazole in sheep naturally infected with *F. hepatica* was a highly efficient treatment. For this reason, Fasciola-infected sheep were treated in the present study by the combined treatment and as several researchers in recent years focused on the metabolic energy deficient effects of fasciolosis [14, 27, 36], the PPARα agonist (2-methyl-2-phenoxy-propionic acid) was associated to the combined treatment in one of the infected groups (group II) in order to evaluate the adjuvant effects on the host metabolism. The World association for the Advancement of Veterinary Parasitology recommends that the treatment efficacy against each genus/species is expressed as highly effective (over 98%), effective (90-98%), moderately effective (80-89%) or insufficiently active (less than 80%) [31]. In this regard, the combined treatment eventually coupled to the PPARα agonist against fasciolosis was considered as “effective” in the present study (the *Fasciola* egg excretion in faeces was reduced by around 95% on day 28) and was in agreement with previous reports [5, 33, 42, 47]. Besides, the PPARα agonist addition to the basal treatment has not significantly improved the treatment efficiency on the parasite elimination.

Anaemia and eosinophilia has been reported as a clinical feature of infection with *F. hepatica* in ruminants [4, 9, 11]. A marked anaemia evidenced by low haemoglobinemia and haematocrit values and not associated with modifications of the cytological erythrocyte characteristics as well as an eosinophilia were also found in infected sheep before treatment in the current study as previously described [9, 29, 37, 38, 47]. A moderate lymphopenia was also present in the beginning of the experiment possibly secondary to bacterial infections. The anaemia seen in the present study may be attributed to the direct blood feeding activity of the flukes and to the haemorrhage into liver parenchyma, bile ducts and the abdominal cavity due to the migrations of immature flukes within the liver [4, 11, 39]. The eosinophilia, occurred as a host response, may be associated with a tissue migratory phase which increases rapidly during the parenchymal stage and persists at a high level after the flukes enter the bile ducts [4, 6, 11, 38]. After the anthelmintic treatment, the eosinophil/leukocyte ratios were immediately decreased and restored whereas the haematocrit, the haemoglobinemia and the lymphocyte population progressively increased and were only partially corrected on day 28. Although it has been reported that lipid peroxidation increases in fasciolosis [24, 40] and that PPARα agonists cause reduction in lipid peroxidation [20], the administration of PPARα agonist in the group II has not here significantly favoured the restoration of the haematological parameters. This situation may be explained by the works of SALEH [40] and ÖZTEZCAN et al. [32]. Indeed, SALEH [40] has previously reported the absence of correlation between the lipid peroxidation intensity in plasma and the anaemia severity in sheep infected with *F. hepatica* and ÖZTEZCAN et al. [32] have found that erythrocytes from rats with liver damage exhibited an increased resistance to the oxidative stress.

On the other hand, parenchyma cell damage in liver due to the migration of immature flukes were reflected in the present study by marked increases in the circulating AST and GDH activities, considered as markers of hepatocyte lesions [6, 14, 15, 36, 38, 40, 47]. As ALT is weakly expressed in the liver of large domestic species [22], the serum ALT activities determined in liver injuries were not remarkably elevated and in the present study like in others [28, 37], fasciolosis was not associated to changes in the serum ALT activity. After the combined triclabendazole/levamisole treatment, the serum enzyme activities significantly declined indicating some compensation in the traumatic damage by liver regeneration [14, 17, 40]. It was also noticed that the serum AST activity was more rapidly rectified (since the 7th day) than the GDH activity which remained significantly depressed until the 14th day. This discrepancy in the 2 enzyme profiles would be related to their tissue-specificity, AST being not an organo-specific enzyme whereas the GDH was considered as a liver specific enzyme, notably in ruminants [22, 39]. Although activation of PPARα in rodent liver and in primary hepatocyte cultures promotes hepatocyte proliferation and regeneration [3, 19], the administration of the PPARα agonist in this study has not seemed to significantly accelerate the liver regeneration, probably because of differences between natural and experimental conditions such as the occurrence of secondary bacterial contaminations during spontaneous infestations.
During fasciolosis, liver damage are also linked to the fluke migration into bile ducts. Increases in the circulating GGT activities in the present study before treatment and in other reports [6, 14, 15, 36, 38, 40] revealed epithelial damage due to penetration of the adult flukes into bile ducts. The progressive decline of this parameter observed during the 28 days long post-treatment period was due to the progressive removal of flukes from bile ducts [6, 14, 17] which occurred with the same intensity in sheep simultaneously treated with the PPARα agonist or not (groups II and I). Although the ALP activity was also considered as a potential marker of cholestatic syndromes, no significant change in the ALP activity according to time and between Fasciola-infected and healthy sheep was recorded in the present study like in others [28, 37] because of a great value dispersion that limits its efficacy for evidencing cholestasis.

The presence of fluke in the bile ducts increases the bile flow without affecting the bile acid secretion in rats and in calves as reported by LOPEZ et al. [28]. By contrast, FERRE et al. [15] showed that both bile flow and bile acid secretion were significantly reduced in subclinical fasciolosis in sheep and consequently the circulating bile acid concentrations were significantly elevated [15]. In the same way, although the bile flow and bile acid secretion were not evaluated in natural infection, this parameter was also significantly increased before treatment in the present study, confirming the bile duct obstruction. After treatment with triclabendazole and levamisole, the serum bile acid concentrations gradually declined, illustrating the efficiency of the antihelminthic treatment, but remained significantly higher than the control values when the PPARα agonist was included in the treatment (group II). This findings are compatible with those of FARINA et al. [13] who reported that PPARα agonist induce cholestasis. Some authors observed hyperbilirubinemia [15, 47], whereas others reported normal bilirubin concentrations [37] in chronic fasciolosis. In the current study, the total bilirubin concentrations have not significantly varied before and after treatment in Fasciola infected sheep. These discrepancies between studies may result from variations in the degree of cholestasis and the duration of infection.

On the other hand, fasciolosis induced damage were associated to reduced liver synthesis capacities. Hypoalbuminemia is stated to commonly occur in liver fluke infections in all host species [4, 9, 11, 22, 38, 39]. Consequently, because of hypoalbuninemia in one hand and hyperglobulinaemia in the other hand, hyper- or hypoproteinaemia [4, 9, 11, 37] as well as a normal proteinemia [39] may occur depending upon the severity and length of infection [9]. The variations of proteinemia and its fractions (albumin and globulins) recorded in the present study were in agreement with previous studies [4, 9, 11, 17, 29, 37, 39] and were roughly identical in triclabendazole/levamisole treated sheep receiving or not the PPARα agonist. Nevertheless, the hyperglobulinemia at the 28th day appeared more pronounced in the group II, because of a probable PPARα positive effect on the liver protein synthesis but no literature information about the PPARα agonist effects on globulin synthesis is available.

Also reflecting the diminished synthesis capacities in liver, the serum glucose concentrations in both infected groups were markedly lowered before treatment, as previously described [15, 36] and would result from depressed hepatic glycogenic pathways and gluconeogenesis during fasciolosis and decreased in voluntary food intake [9, 15, 27, 36]. As migrating flukes cause a severe liver injury and the death of lot hepatocytes [14, 37], the liver glycogen reservoirs were dramatically reduced. Moreover, FERRE et al. [14] demonstrated that the decrease in food intake accompanying fasciolosis is related to the liver injury. After the antihelminthic treatment in the present study, the serum glucose concentrations progressively increased, suggesting that exogenous and endogenous glucose supplies were positively correlated to the fluke elimination and to liver regeneration. As the improvement in the glucose metabolism was earlier (on days 7 and 14) in sheep receiving also the 2-methyl-2-phenoxy-propionic acid, it can be concluded that the PPARα agonist positively acts in the glucose homeostasis, and in particularly promotes the gluconeogenesis [13, 23, 34, 41] and may be useful in restoring negative energy balance during fasciolosis.

Despite the reduced liver synthesis capacities due to the fluke induced liver damage, the serum triglyceride and total cholesterol concentrations as well as the total lipidemia were only slightly (and not significantly) decreased in infected sheep before treatment compared to the control values. However, the LDL associated cholesterol concentrations (LDLc) were dramatically depressed while the HDL associated cholesterol concentrations (HDLc) remained unchanged. These findings were in accordance with a previous report [21] and suggest that lipid catabolism and notably the fatty acid oxidation would be preserved and/or promoted during fasciolosis in order to counteract the negative energy balance. Indeed, OSMAN et al. [31] observed a decrease in the circulating LDL concentrations in human fasciolosis which may be attributed to the degenerative necrotic damage in hepatocytes and an increase in the circulating VLDL concentrations which may be compatible with a preserved triglyceride distribution throughout the organism and secondary fatty oxidation in peripheral tissues. Nevertheless,
whereas OSMAN et al. [31] also noted a decrease in the HDL concentrations in human fasciolosis, this parameter was not significantly affected here and even tended to slightly increased in infected sheep before treatment compared to the control healthy sheep. This discrepancy would result in one hand from the enhancement of cholesterol and some fatty acid transport from peripheral tissues to liver in order to provide cholesterol necessary to the bile acid formation and to assure energy supply, respectively and in the other hand, to the genetically determined high HDL synthesis in animals (contrary to humans) particularly during fasting state [22] which is associated to fasciolosis [14].

After the anthelmintic treatment, whereas the total lipidemia has poorly varied according to time and to the treatment type, the serum triglyceride concentrations gradually still declined and reached minimal values at the 28th day in both treated groups and the total cholesterolemia significantly increased according to time compared to the pre-treatment values and was maximal at the end of the experimental period. Additionally, cholesterolemia in the group treated with the combined treatment (triclabendazole /levamisole + PPARα agonist) became significantly higher /levamisole + PPARα agonist) became significantly higher compared to the control values and than values observed in the group I. In parallel, whereas the LDLc weakly increased but remained significantly depressed during the post-treatment period, particularly in the group II at the 28th day, the HDLc concentrations increased and significantly exceeded the control values only on day 28 in the group I and since the 7th day in the group II. The continued decrease in the triglyceride concentrations would be related to changes in energy supply induced by the anthelmintic treatment: as the endogenous glucose supply was partially restored in liver, the triglyceride distribution via VLDL may gradually decline. However, because the glycolysis supply was still insufficient, the energy balance remained still negative and was aggravated by drop in triglyceride exportation from the liver. In addition, it was noted that the administration of the PPARα agonist has not exerted any significant effect on the triglyceride concentrations in accordance with previous reports [7, 18, 30, 35]. In the same way, the progressive increase in the LDLc concentrations suggests that the liver functions in cholesterol synthesis and esterification would be partially recovered [31]. Nevertheless, the PPARα agonist administration in this study has minimized this effect, probably because of a negative impact on the LDL synthesis [7, 10, 30]. The increase in the HDLc concentrations indicated that the cholesterol transport from peripheral tissues to liver was exacerbated for removal of negative energy balance in fasciolosis and the administration of the PPARα agonist has strengthened this mechanism as already shown in humans [7, 10, 30]. Moreover, KERSTEN et al. [23] have reported that PPARα agonist stimulates hepatic fatty acid oxidation to supply substrates that can be metabolized by other tissues.

As a conclusion, the present study explored for the first time the potential metabolic effects of a PPARα agonist used in the fasciolosis treatment in sheep. Although this compound has not directly improved the liver regeneration, it has significantly and positively stimulated promoting in this way the recovery of negative energy balance. For this reason, the administration of PPARα agonist in addition to the anthelmintic treatment in natural fasciolosis may contributed to correct the metabolic alterations and to reduce the inherent economical losses.

References

12. EZAKI H., YOSHIDA Y., SAJI Y., TAKEMURA T., FUKUSHIMA J., MATSUMOTO H., KAMADA Y.,
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