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# Identification of somatic proteins in *Haemonchus contortus* infective larvae ($L_3$) and adults

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**Summary**

*Haemonchus contortus* is considered the most pathogenic nematode in sheep production systems based on grazing. Comparing infective larvae ($L_3$) with adult parasites can lead to the identification of proteins that play an important role in parasite-host interactions. In this study, we report a list of *H. contortus* somatic proteins and made a comparative analysis of somatic proteins of $L_3$ and adult worms. $L_3$ and adult parasites were subjected to protein extraction and subsequently to peptide fractionation. Peptides were analysed by mass spectrometry and LC-MS/MS data analysis. Data analysis and search on SEQUEST and MASCOT against *H. contortus* from the WormBase ParaSite database resulted in the identification of 775 unique peptide sequences corresponding to 227 proteins at 1 % FDR. From these, 18 proteins were specific to $L_3$ and 63 to adult parasites. The gene ontology (GO) enrichment analysis of the proteins specific to $L_3$ and adult worms to gain insight into cellular components, molecular functions and biological processes that affect the parasite-host interaction showed some differences between the two parasite stages. The list of proteins found provides a database to identify target proteins that could be useful as biomarkers of the infection or in the generation of anthelmintic drugs that inhibit proteins essential for the establishment of the infection and the survival of adult parasites. They can also serve as new candidates for vaccine research.
**Keywords:** Gastrointestinal parasites; biomarkers; ewes; tropical production

## Introduction

Gastrointestinal (GI) parasites are one of the main causes of diseases, death and economic loss in small ruminant production. *Haemonchus contortus* has been considered the most pathogenic nematode in production systems based on grazing (Bordoloi *et* al., 2012; Arsenopoulos *et al*., 2021).

Since anthelmintic resistance has been described in several species of parasites (Pandey and Sivaraj, 1994; De Albuquerque *et al*., 2017), new strategies to control the infections and their adverse effects have been developed. Among these, the selection of animals with resistance to GI parasites has been in the spotlight (Palomo-Couoh *et al*., 2017; Aguerre *et al*., 2018; Estrada-Reyes *et al*., 2019; Zaragoza-Vera *et al*., 2019 ). Thus, the study of protein molecules (involved mainly in secretion-excretion) focused on the parasite-host interactions and the identification of targets to

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elaborate new drugs or vaccines (Gadahi *et al*., 2016; Wang *et al*., 2019a). Some studies concerning the genome, transcriptome and secretome of *H. contortus* have been conducted (Yatsuda *et al*., 2003; Laing *et al*., 2013; Gadahi *et al*., 2016; Lu *et al*., 2020).
The somatic proteins of *H. contortus* were recently reported in a study (Wang *et al*., 2019b), while, their biological implications and particular functions have been scarcely discussed. In this line, comparing infective larvae ($L_3$) with adult parasites can lead to the identification of proteins that play an important role in diverse parasite-host interactions, such as the biological mechanisms involved in the establishment of the infection and the survival of the parasite, evasion of the immune response, immunogenic protein recognition by the host, metabolic functions, and other interactions that could improve the understanding of this parasitic infection.
Proteomics studies have contributed to elucidate the role of somatic proteins in the modulation of cellular immune response against *H. contortus* (Laing *et al*., 2013; Gadahi *et al*., 2016; Naqvi *et al*., 2020; Tian *et al*., 2020). Somatic proteins such as HC23 and their recombinant version (rHC23), rHcp26/23 and recombinant galectin (rHco-gal-m and rHco-gal-f) have been used for inducing partial protection against of *H. contortus* infection (Yanming *et al*., 2007; García-Coiradas *et al*., 2010; González-Sánchez *et al*., 2018).
In the same way, a potential use of *H. contortus* somatic proteins could be to generate immunodiagnostic antigens to identify early and late *H. contortus* infections in sheep, similar to the reported in goats (Naqvi *et al*., 2020), or as an alternative method to identify specific antibodies of resistant sheep that contribute with the traditional method of faecal egg count (FEC) to identify resistant animals (Palomo-Couoh *et al*., 2016; Palomo-Couoh *et al*., 2017; Cruz-Tamayo *et al*., 2020).
In this study, we report a list of somatic proteins of *H. contortus* and a comparative analysis between somatic proteins from $L_3$ and adult parasites recovered from an infected lamb.

## Materials and Methods

*Experimental infection with H. contortus*
The infective larvae of *H. contortus* were initially obtained from a laboratory strain donated by the National Center of Disciplinary Investigation in Veterinary Parasitology (CENID-PANVET). This strain was used to infect a male Pelibuey lamb, raised free of GI parasites.
The infection was carried out when the lamb reached 2.5 months of age (11.1 kg live weight, LW). The infection was performed using an oral dose of 400 $L_3$/kg LW. $L_3$ were obtained from coprocultures made of faeces of the infected lamb and weekly collected starting at 21 days after infection, according to the Corticelli and Lai technique (1963).
On day 35 post-infection, faeces were collected to calculate FEC. Subsequently, the lamb was sacrificed following the official national standards (Norma Oficial Mexicana NOM-033-ZOO-1995) to collect adult parasites directly from the abomasum during the necropsy. Parasites were washed with phosphate buffer solution (PBS, 0.01 M, pH 7.4) to eliminate cellular detritus. Afterwards, parasites were frozen at -20 °C until use.

*Parasitological evaluation of the experimentally infected lamb*
Faecal samples were taken directly from the rectum of the lamb and collected in polyethylene bags. The faecal samples were processed by modifying the McMaster technique to determine FEC. The number of eggs per gram of faeces (EPG) was adjusted with a correction factor of 50 (Cringoli *et al*., 2004). Moreover, coprocultures were performed according to Corticelli and Lai (1963) to obtain *H. contortus* $L_3$ from the infected lamb. The infective larvae genus was determined according to Van Wyk and Mayhew (2013).

*Protein extraction*
The $L_3$ and adult parasites were pulverised to a fine powder with the mortar and pestle technique at liquid nitrogen temperature. Pulverised samples were resuspended in 500 µl of protein extraction buffer (500 mM Tri-HCl, pH 7.5, 100 mM KCl, 100 mM sucrose, 50 mM EDTA, 50 mM DTT, 1 mM PMSF and 1X complete Roche protease inhibitor cocktail). Proteins were recovered from the clarified homogenate by precipitation using a solution of TCA/acetone at 20 % cold at a volume proportion of 1:1 followed by two washes with 80 % acetone solution (Niu *et al*., 2018). Protein pellets were air-dried for 3 min and kept at -20 °C. Then, they were resuspended in 300 µl of 50 mM ammonium bicarbonate solution. Subsequently, the protein concentration was determined by the Peterson method using 10 µl of the extract and bovine serum albumin protein as reference (Peterson, 1977).

*In-solution digestion and peptide fractionation*
The protein content of 100 µg was reduced with 15 mM DTT for 1 h at 25 °C followed by alkylation with 30 mM iodoacetamide dithiothreitol for 1 h at 25 °C in darkness. Subsequently, protein samples were quenched with 30 mM DTT for 10 min at 25 °C in darkness. Trypsin Gold (Promega™, Madison, Wisconsin, USA) was added at a ratio of 1:30 trypsin/protein (w/w). Afterwards, protein digestion was carried out for 16 h at 37 °C, and more trypsin was added at a ratio of 1:30 trypsin/protein (w/w). The samples were left to incubate for 4 h at 37 °C. Digestion was quenched by making samples with 1 % formic acid that were dried using a SpeedVac Vacuum Concentrations (Thermo Fisher Scientific Inc, Waltham, Massachusetts, USA) and stored at -70 °C until fractionation of peptides using Strong Cation Exchange (SCX) chromatography.
Peptides were resuspended in equilibration buffer consisting of 5 mM KH2PO4 (pH 3)/25% acetonitrile (ACN) for SCX fractionation using a 50 mg sorbent HyperSep ™ SCX cartridge (Thermo Fisher Scientific Inc, Waltham, Massachusetts, USA). The SCX cartridge was conditioned with 2 ml milli-Q water, washed with 1 ml of 10 mM KH2PO4 (pH 3)/25% ACN and subsequently washed twice with 2 ml of milli-Q water. The cartridge was placed in equilibration buffer before sample loading. After three washes with 1 ml

of equilibration buffer, peptides were eluted in a stepwise gradient of increasing salt concentrations (75, 250 and 500 mM KCl) in equilibration buffer. Eluted peptide fractions were then dried in SpeedVac Vacuum Concentrations (Thermo Fisher Scientific Inc, Waltham, Massachusetts, USA). The resulting peptides were resuspended in 1 ml of 0.1 % formic acid for desalting using 25 mg HyperSep C18 cartridges (Thermo Fisher Scientific Inc, Waltham, Massachusetts, USA). Eluted peptides were then desalted using Zip-Tip pipette tips (Millipore, Billerica, Massachusetts, USA). Desalted peptides were vacuum-dried and resuspended in 0.1 % formic acid for liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis.

*Mass spectrometry and LC-MS/MS data analysis*

The desalted peptide samples were analysed in an LC-MS/MS system consisting of EASY-nLC 1000 nano-HPLC (Thermo Fisher Scientific Inc, Waltham, Massachusetts, USA) coupled to an LTQ-Orbitrap Elite mass spectrometer (Thermo Fisher Scientific Inc, Waltham, Massachusetts, USA). Chromatographic separation was performed using a reversed-phase Acclaim PepMap-100 trap column (20 × 0.075 mm i.d., 3 µm particle size, 100 Å pore size; Thermo Fisher Scientific Inc, Waltham, Massachusetts, USA) coupled to an EASY-Spray C18 analytical column (150 × 0.075 mm i.d., 2 µm particle size, 100 Å pore size; Thermo Fisher Scientific Inc, Waltham, Massachusetts, USA) at 40 °C with a flow of 300 nl $min^{-}1$, from 5 % to 40 % ACN in 0.1 % formic acid, in a 60 min gradient. The mass spectrometer in data-dependent acquisition mode and the Orbitrap™ analyser (Thermo Fisher Scientific Inc, Waltham, Massachusetts, USA) were used. Full scan MS spectra were acquired with a resolution of R = 12,000 followed by MS/MS acquisition with a resolution of R = 15,000. The 15 most intense precursor ions from the full scan were fragmented using higher-energy C-trap dissociation. Fragmented masses were excluded for 90 s. Three independent biological replicates were performed. Carbamidomethylation was used as fixed modification.

The mass spectrometry data files were processed using the Proteome Discoverer 2.2 software (Thermo Fisher Scientific Inc, Waltham, Massachusetts, USA), and protein identification was performed with the MASCOT search engine (Matrix Science™) and the SEQUEST™ software (Eng *et al*., 1994). A total of 24,642 amino acid sequences were retrieved from the WormBase ParaSite database (Laing *et al*., 2013) and used for spectra matching. The database search allowed static carbamidomethylated cysteine residues (+57.02 Da), dynamic oxidised methionine residues (+15.99 Da), up to two missed trypsin cleavages, a tolerance of 10 ppm for precursor ions and 0.6 Da for fragmented ions. The masses of both precursor and fragmented ions treated as monoisotopic and multiple charges were considered. The data were filtered using an estimation of 1 % false discovery rate (FDR) according to a previous study (Rohrbough *et al*., 2006). The determination of protein abundance was evaluated by spectral counts (Arike & Peil, 2014).

**Ethical Approval and/or Informed Consent**

This study was approved by the Ethical Committee of University in State of Yucatan, Mexico (Approval ID: CB-CCBA-D-2019-004). The animal used in this study, biological samples and residues were treated and handled in accordance with federal regulations in Mexico (NOM-046-zoo-1995, NOM-087-ECOLSSA1-2002) and following the recommendations emitted by Ethical Committee.

**Results**

The lamb infected with *H. contortus* ($L_3$) presented an FEC of 7, 850 EPG at 35 days after inoculation. The necropsy revealed 634 adult specimens of *H. contortus* that were directly collected from the abomasum of the infected lamb and used for total protein extraction.

A comparative analysis between $L_3$ and adult worms was performed after protein identification. $L_3$ showed a protein profile different from the adult parasites. Data analysis and search on SEQUEST and MASCOT against *H. contortus* from the WormBase ParaSite database resulted in the identification of 775 unique peptide sequences corresponding to 227 proteins at 1 % FDR (Supplementary Table S1). From these, 18 proteins were specific to $L_3$ and 63 to adult parasites.

We performed gene ontology (GO) enrichment analysis of the proteins specific to $L_3$ and adult worms to gain insight into cellular components, molecular functions and biological processes that affect the parasite-host interaction. In the $L_3$ stage, the GO category of cellular components included the following: transcription complex, intracellular and extracellular region, intracellular organelle and non-membrane bound organelle (Fig. 1). The molecular functions category included binding (e.g., heterocyclic binding, organic cyclic compound binding, carbohydrate derivative binding) and catalytic activity (hydrolase, transferase and catalytic activity acting on a protein). The biological functions category included metabolic processes (primary and organic and nitrogen compound metabolic process) and protein folding.

In the adult worm stage, the GO category of cellular components included intracellular organelle, non-membrane bound organelle, the endomembrane system, the nuclear outer membrane-endoplasmic reticulum membrane network, and protein complexes such as myosin complex, catalytic complex and proteasome core complex (Fig. 1). As for the molecular functions category, it included catalytic activity (transferase, hydrolase, lyase, oxidoreductase, and catalytic activity that acts on protein), binding (ion, organic cyclic, heterocyclic compound binding, carbohydrate derivative and drug binding), and structural constituents of the cytoskeleton and ribosome. The biological processes category included active metabolic processes linking organic substances, primary metabolic processes, nitrogen metabolic processes, oxidoreduction processes, and cellular processes such as microtubule-based processes and regulation of cellular processes. The comparison between the

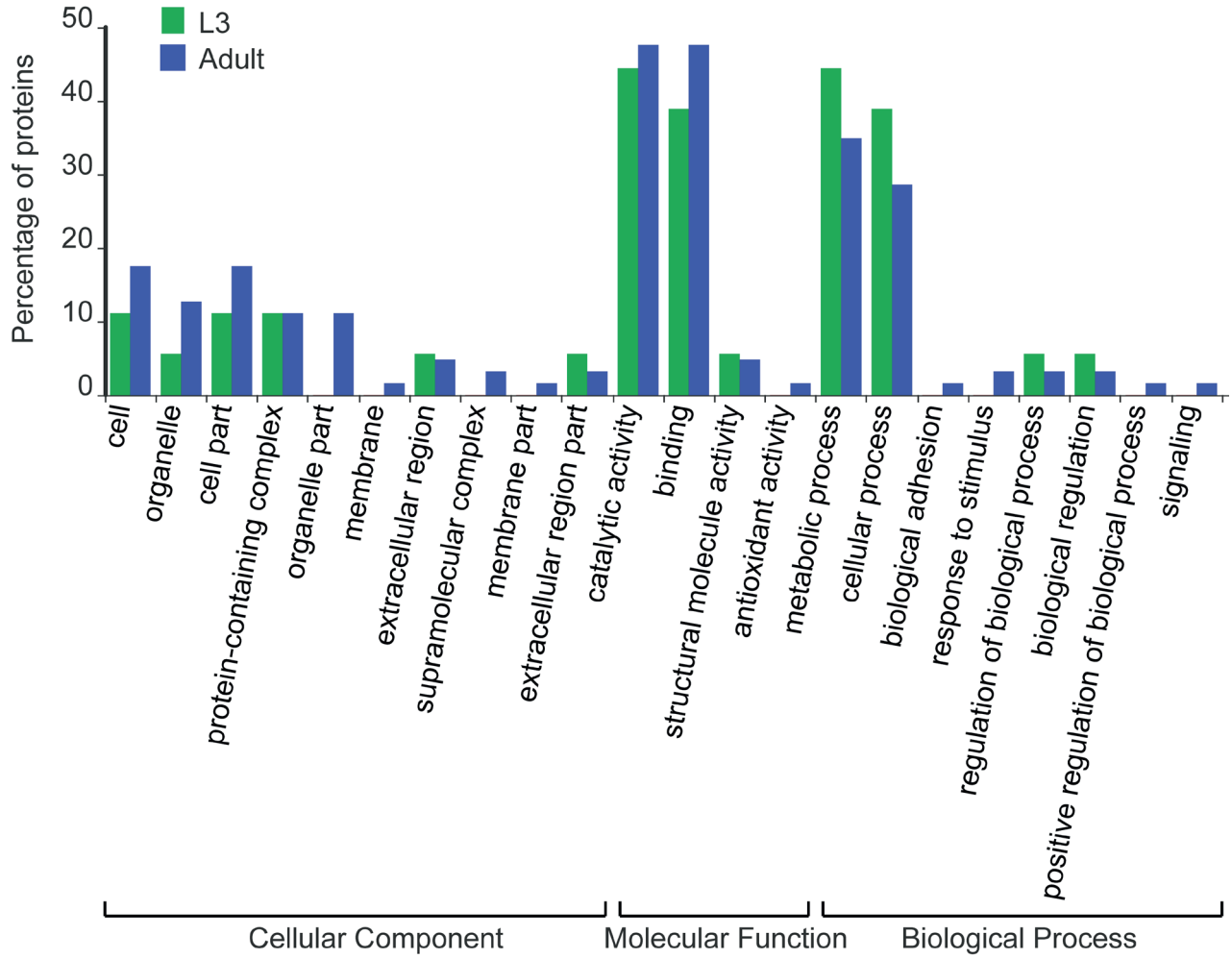


Fig. 1. Comparative analysis of gene ontology from proteins identified in $L_3$ and adult worms of *Haemonchus contortus*. Proteins were classified according to cellular component, molecular function, and biological process.

GO categories of adult worms and $L_3$ suggests that the adult stage implicates an array of enzymatic activities, structural changes and degradation of proteins.

To identify significant differences in protein abundance between $L_3$ and adult parasites, the altered proteins were defined with spectral counts that were normalised across $L_3$ and adult parasites by calculating Z-score values for each protein and with a significance level of $p < 0.001$. As result, 52 from the 219 proteins showed different abundance between $L_3$ and adult parasites (Fig. 2). Proteins accumulated in adults include vitellogenin (CDJ88321.1), proteases (AAC03561.1, CDJ97529.1, CDJ87267.1 and AAV68383.1), ubiquitination component (CDJ87593.1), heat shock proteins such as HSP20 (CDJ94402.1), HSP70 (AEO14648.1 and CDJ90614.1) and HSP90 (ACU00668.1), key metabolic proteins such as phosphoenolpyruvate carboxykinase (CDJ86804.1), isocitrate lyase phosphoryl mutase and malate synthase (CDJ95172.1), 6-phosphogluconate dehydrogenase (CDJ88445.1), glutamate dehydrogenase (ACT34055.1), acetyl-CoA hydrolase transferase (CDJ95142.1), thioredoxin, and structural proteins such as actin (CDJ93106.1) and myosin (CDJ95284.1). Collectively, the above-identified proteins suggest that scavenging of reactive oxygen species (ROS), protein folding and degradation, and metabolic changes are implicated in the parasite-host interaction.

## Discussion

The immune response against GI parasites is considered the main factor associated with the resistance level of the sheep (Aguerre *et al*., 2018; Estrada-Reyes *et al*., 2019 ). This response is toward different parasite proteins, either secretory-excretory products or structural compounds (Gadahi *et al*., 2016; Sakthivel *et al*., 2018; Lu *et al*., 2020). The secretory-excretory products have been widely studied due to functions such as tissue penetration and their capability to degrade host proteins (Cox *et al*., 1990; Yatsuda *et al*., 2003; Wang *et al*., 2019a). Moreover, some secretory-excretory products also establish the infection because they regulate the

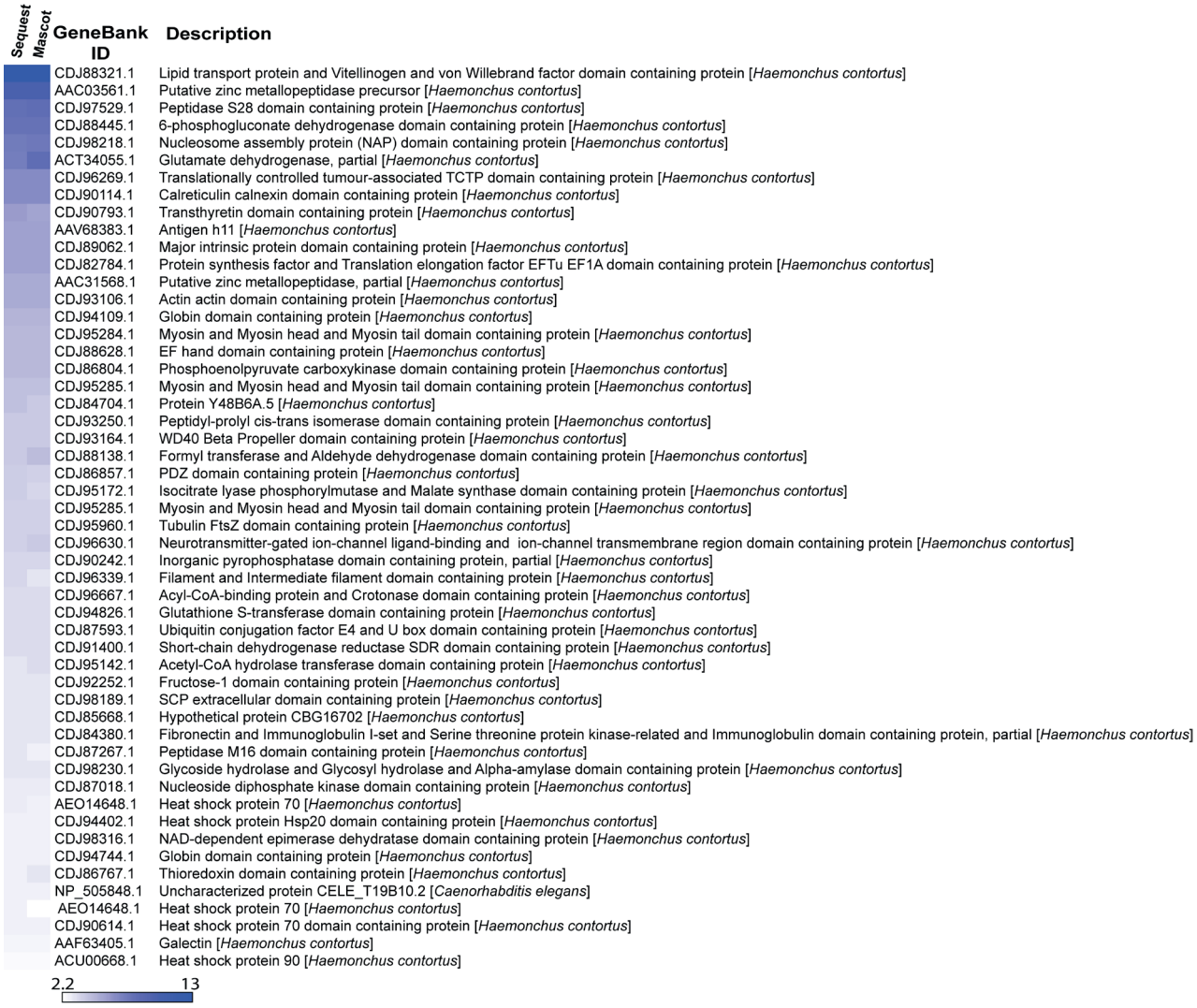


Fig. 2. Heat map of spectral counts for 52 differential accumulated proteins.
Sequest and mascot searches with a 1 % protein FRD and P-value of 0.001 for Z-score are reported.

immune response of the host, facilitating the immune evasion of the parasite that induces chronicity related to a T cell-mediated hyporesponsiveness (Lu *et al*., 2020).

Somatic proteins also play an important role in the immune response against GI nematodes due to which some of them have been evaluated as vaccine candidates (Sakthivel *et al*., 2018; Wang *et al*., 2019b). Thus, a study reported that proteins such as rHcp26/23 have a protective effect only with challenges against low and moderate parasite burden (García-Coiradas *et al*., 2010). The immunization of lambs using the recombinant protein rHc23 produced low of egg excretion and low parasites burden (Gonzalez-Sanchez *et al*., 2019). However, highest protection of lambs was achieved using aluminum hydroxide as adjuvant and 200 µg/dose rHc23 reaching a reduction of over 70 % of the abomasal parasitic burden (Gonzalez-Sanchez *et al*., 2019). The recombinant protein rHc23 (23 kDa) was patented for induce more than 85 % of immunity in lambs (Alunda *et al.,* 2014). However, the efficacy of the immunization in lambs seems to depend of the parasite burden used as challenge or by the use of adjuvants (Fawzi *et al*., 2014; González-Sánchez *et al*., 2018, 2019). In other study, recombinant HcGAPDH protein expressed on probiotic *Bacillus subtilis* spores protected sheep from *Haemonchus contortus* infection by inducing both humoral and cellular responses which reduced the eggs excretion in feces by 84.1 % and adult worms by 71.5 % (Yang *et al*., 2020). Other somatic protein of *H. contortus* used as vaccine antigen is actin although the parasite burdens were reduced only 34 % in vaccinated animals (Yan *et al*., 2014). The HcGAPDH and actin were found in protein extracts from $L_3$ and adult parasites in present study,

On the other hand, it is known that gut antigens of *H. contortus* (H-gal-GP) inoculated in lambs induce an immune response mediated by CD4 and T lymphocytes, reducing the FEC in the infected

animals (Karanu *et al*., 1997). The main components of H-gal-GP are metalloproteases and pepsinogen-like aspartyl proteinases that *H. contortus* use to digest the ingested blood (Scarff *et al*., 2020). H-gal-GP and gut-derived antigens H11 (aminopeptidases) were antigens with good vaccination response against *H. contortus* and therefore, they were used in the commercial Barbervax™ vaccine (Nisbet *et al*., 2016). Despite its effectiveness, the vaccine has disadvantages such as the lack of the global availability of the product; repeated vaccination is required to stimulate high amount of antigen-specific circulating antibody levels and special management of peri-parturient ewes and young lambs are limiting of the vaccine (Nisbet *et al*., 2016; Bassetto *et al*., 2018; Kebeta *et al*., 2021). A vaccine targeting multiple stages of *H. contortus* ($L_3$ surface antigens and gut antigens of $L_4$ and adult stages) has been proposed (Nisbet *et al*., 2016) and likely, the integration with recombinant proteins such as 15- and 24-kDa ES proteins, galectins, Hc 23, enolase and cysteine could improve the protective immunity inclusive in young lambs, peri-parturient ewes or in animals with infections with high parasite burdens.

Somatic proteins of *H. contortus* have also been used as somatic antigens for immunodiagnosis of the infection (Javare, 2017). The recombinant *H. contortus* adhesion-regulating molecule protein (rHCADRM) was evaluated as potential antigen for diagnosis of early infection in goats. The results showed that rHcADRM can be recognized in the serum as early as 14 post-infection days and antibodies were maintained for over 89 days. The ELISA test developed using this recombinant protein obtained a sensitive (90.6 %) and specific (93.75 %), no showing cross-reaction with other common parasites, which places it as an important antigen for diagnosis of *H. contortus* infection in goats. Similarly, recombinant cold shock domain containing protein (rHc-CS) was evaluated by immunoblotting and ELISA assays during early and late infections of *H. contortus* in goats. Results showing specific antibodies from 14 days to 103 post-infection days and specificity and sensibility values of 100 % in both techniques (Naqvi *et al*., 2020b). However, the use of these recombinants proteins should be validated in sheep.

In our study, a long list of proteins and their differences between the $L_3$ and adult stages are presented. We focused our attention on $L_3$ and adult parasites that live in mammalian hosts. Both the $L_4$ and adult stages utilise catabolic proteins especially in degradation of haemoglobin, meaning that several of these proteins should be present in these parasitic stages (Wang *et al*., 2019b). In the present study, peptidase S28, putative zinc metallopeptidase precursor, peptidase C1A, peptidase M13 and proteinase inhibitor I25 were present only in the adult stage, whereas proteinase inhibitor I33, aminopeptidase, ubiquitin conjugation factor E4, gelsolin, calpain clp-1, peptidase M16 and peptidase M12A were present only in the $L_3$ stage. These findings show that the catabolism of proteins is carried out using different enzymes according to the developmental stage.

Differences in proteins involved in the metabolism of carbohydrates and lipids were found in $L_3$ and adult parasites, in line with the report by Wang *et al*. (2019b). In the present study, enzymes involved in the catalysis of ATP, such as adenylyl-sulfate kinase, ATP-sulfurylase domain-containing protein, Fructose-1 domain-containing protein and ATP synthase subunit delta, were not found in the $L_3$ stage. However, it is known that $L_3$ stage synthetizes glycogen from lipids and them are able to degrade carbohydrates via Krebs cycle (Harder, 2016). Therefore, many of the enzymes involved in glycolysis are not present in this stage, as shown in this study.

Controversial findings were observed in the case of enzymes involved in lipid metabolism. Acetyl-CoA hydrolase, acyl-CoA-binding protein and crotonase domain-containing protein were found only in $L_3$. Similar results were previously found in the $L_3$ stage of *H. contortus* (Wang et *al.,* 2019b). Therefore, the assumption of Wang *et al.* (2018), arguing that *H. contortus* alters its lipidome by adapting metabolism to take advantage of the fatty acids in the blood of the host, seems to be true. Additionally, the protein dhs-3 involved in several lipid functions, such as storage and transportation, metabolic regulation and serving as a source of membrane lipid precursors through cellular organelles named “lipid droplets” (organelles constituted by a neutral lipid core covered by a monolayer of phospholipids and proteins), were exclusively found in $L_3$, suggesting that lipid metabolism has different routes in $L_3$ and adult parasites (Xie *et al*., 2019). Similarly, thiolase, an enzyme catalysing acetyl-CoA, was present only in the larval stage, in agreement with the peaks of the transcriptional levels of its encoding gene Hc-daf-22 in *H. contortus* $L_3$ and $L_4$, suggesting an important role in these larval stages (Guo *et al*., 2016).

Other proteins found exclusively in $L_3$ in this study were vinculin, alpha-catenin, calsequestrin, proteinase inhibitor I33, macrophage migration inhibitory factor, catalase, galectin and calponin. These proteins seem to have an important role in the structure and motility of the parasite. Vinculin and alpha-catenin form a complex of proteins named cadherins that contribute to cellular adhesion and contractibility, both proteins are important in the motility of the parasite (Dufour *et al*., 2013). The presence of calsequestrin and calponin, involved in $Ca^{2+}$ regulation and highly related to myosin and actin in the tissue contraction (El-Mezgueldi, 1996; Wang & Michalak, 2020), reinforces this assumption.

The regulation of the initial immune response in the host seems to be directed by $L_3$ and $L_4$ stages of *H. contortus* and several structural proteins are involved in this process. Proteinase inhibitor I33 inhibits trypsin and anti-coagulation of blood, favouring in this way the feeding and the survival of $L_4$ (Yi *et al*., 2010). The macrophage migration inhibitory factor is a cytokine that regulates the inflammatory response (Lang *et al*., 2018). Similarly, galectin modulates host-pathogen interactions (Sakthivel *et al*., 2018). Its functions involve modulating the production of Th2 and Th9 cells, IL-4, IL-9, blood mononuclear cell proliferation, nitric oxide and cell migration in the host (Naqvi *et al*., 2020).

Catalase is an enzyme with antioxidant effects, used by *H. con-*

*tortus* as a defence against hydrogen peroxide and other oxygen reactive species produced by phagocytes (e.g., macrophages, eosinophils and neutrophils) of the host (Kotze, 2003). Notably, in our study, catalase was only present in $L_3$ but not in the adult stage. Although catalase effects were previously studied in the larval and adult stages of *H. contortus* (Kotze & McClure, 2001; Kotze, 2003), this effect was indirectly measured by monitoring the disappearance of hydrogen peroxide after the addition of glucose oxidase in cultures of $L_4$ and adult *H. contortus*. Therefore, in these studies, the presence of catalase in both parasitic stages was assumed but not directly determined.

In general, our findings show that protein abundance was high in adult parasites. However, most proteins identified in both stages involved the categories of catalytic activity, binding, metabolic and cellular processes.

Some proteins found in the present study were not found in previous reports of the *H. contortus* proteome (C05D9.9, isoform b, T10G3.3, C29F5.1, dhs-3, DUF148 domain-containing protein, Y48B6A.5 and p15). Moreover, the functions of the unnamed protein products such as VDO47405.1, CDJ93528.1 and VDO21904.1 should be elucidated in future research to contribute to identifying them.

In the present study, specific proteins of the $L_3$ and adult stages were identified and compared with the purpose of understanding the biological processes involved in the establishment of infection and the role of somatic proteins in the survival of the parasite. The list of proteins provides a database that can be used to identify target proteins that may serve as biomarkers of the infection, to generate anthelmintic drugs that inhibit proteins essential for the establishment of the infection and the survival of adult parasites, or to introduce new candidates for vaccine research. Future studies should be performed to discover the functions of unknown proteins of *H. contortus* until today, as well as to unravel the recognition and specific role of somatic proteins in the immunological responses generated in the infected sheep.

**Conflict of Interest**

The authors declare no conflicts of interest.

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Supplementary Table S1. The full list of somatic proteins found in $L_3$ and adults of *Haemonchus contortus* is available online at https://doi.org/10.2478/helm-2022-0017.