Effect of a feed additive containing yeast cell walls, clove and coriander essential oils and *Hibiscus sabdariffa* administered to mid-lactating dairy cows on productive performance, rumen fluid composition and metabolic conditions

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**ABSTRACT**

Thirty-six multiparous Holstein mid-lactating cows were housed in 6 pens (6 cows/pen) and allocated in two groups (3 pens/group), receiving a control total mixed ration (CTR), or the same diet supplemented with a feed additive (TRT). Between 15 and 35 days from enrolment (DFE) the dry matter intake (DMI) of each pen, and the individual milk yield (MY), rumination time (RT), the compositions of milk and rumen fluid and the metabolic profile of plasma were monitored regularly. At 35 DFE, cows started a 1-week wash-up period prior to the changeover. Data were analysed with a mixed model using repeated measures. Cows receiving the additive had higher DMI and MY (*p* < .01), a longer RT between 22 and 29 DFE (*p* < .05), a higher total VFA concentration and a lower pH in the rumen fluid (*p* < .01 and *p* = .04, respectively). At 15 DFE, TRT cows had a higher concentration of lactose in their milk, and lower concentrations of BHB, ceruloplasmin and haptoglobin, paired with higher concentrations of albumin, paraoxonase and aspartate aminotransferase in their plasma (*p* < .01). These outcomes suggest a positive effect of the additive on increasing DMI and MY, possibly reflecting that it had a combined action on the rumen and the liver.

**HIGHLIGHTS**

- The additive increased feed intake, rumination time and milk yield.
- It improved rumen fermentations and liver activity, and mitigated ketogenesis and inflammatory conditions.
- Beneficial actions of the active components on rumen and liver functions could be hypothesised.

**Introduction**

Rumen and liver function are two main factors that impact the efficient use of nutrients by dairy cows. Increasing the efficiency of feed use is the main goal of dairy nutritionists, to achieve greater milk yields, and to improve milk quality (Bertoni et al. 2001). The inclusion of feed additives in the diet represents a promising strategy to achieve this goal through modulating rumen fermentation and animal metabolism. In this respect, feed additives which create synergies among rumen bacteria, through enhancing or inhibiting specific microbial populations, has been proposed as a strategy to improve feed use (Calsamiglia et al. 2006; Jouany 2006). Several feed additives currently available contain active components that interact directly with cow’s metabolism (i.e. yeasts cell walls, phytoextracts), stimulating positive responses in several compartments (the rumen and the liver, among the others) and improving cow health (Mezzetti et al. 2020; Lopreiato et al. 2020).

Yeast cultures are known for their ability to prevent subclinical acidosis, by acting directly in the rumen...
(Chaucheyras et al. 1996; Rossi et al. 2004) and improving feed intake of animals (Carro et al. 1992; Chaucheyras-Durand and Fonty 2001). Further, several plant essential oils exert beneficial actions on rumen functions through selectively inhibiting rumen bacterial, protozoal, or fungal populations (Tan and Vanitha 2004; Barnes et al. 2005). *Hibiscus sabdariffa* L. (HS, roselle; Malvaceae) is currently used as a fodder for ruminants in tropical countries (Da-Costa-Rocha et al. 2014). Its effects on metabolism and performance of dairy animals have never been investigated, but in human medicine, several beneficial properties have been attributed to phenolic acids, organic acids and anthocyanins contained in HS extracts (Da-Costa-Rocha et al. 2014). Among others, these include anti-fungal and antibacterial activities (Da-Costa-Rocha et al. 2014), suggesting a possible role of HS in modulating rumen fermentation (Dorman and Deans 2000; Burt 2004). Furthermore, HS extracts are known to modulate lipid metabolism and have antioxidant and anti-inflammatory properties, acting as a nephroprotective, hepatoprotective, anti-diabetic and anti-hypertensive agents (Dafallah and Al-Mustafa 1996; Usoh et al. 2005; Maganha et al. 2010). As oxidative stress, systemic inflammatory conditions and dysregulated lipid metabolism are known to be the main cause of altered liver function in dairy cows (Celi 2011; Graugnard et al. 2013; Bertoni and Trevisi 2013), the properties of HS suggest its use as a feed additive might be a promising strategy to improve liver metabolism in ruminant animals.

The feed additive AL878 Valkalor® (IDENA, Sautron, France) contains a mixture of derivative products of yeasts, a blend of clove and coriander essential oils, HS calyxes’ powder, calcium carbonate, sodium sulphate, sepiolite, zinc oxide and colloidal silica. Although the effect of this feed additive on lactating dairy cows has not been evaluated to date, to the best of our knowledge, a positive effect on feed utilisation could be hypothesised based on the components included in the commercial formula. In the present study, the commercial feed additive Valkalor was fed to dairy cows at mid-lactation to assess its effect on feeding behaviour (feed intake and rumination time), milk yield and metabolic conditions. The hypothesis of the study was that the mixture of derivative products of yeasts, blend of clove and coriander essential oils and HS calyxes’ powder contained in Valkalor could improve the performance of dairy cows, by acting on rumen fermentation and ameliorating unproductive liver functions.

### Materials and methods

#### Experimental design and animal management

The trial was carried out at the Università Cattolica del Sacro Cuore research dairy barn (Cer zoo Experiment Station, San Bonico, Piacenza, Italy), from October to January 2015. A group of 36 Holstein dairy cows [number of lactations: 1.9 ± 1.5; body weight (BW): 622.6 ± 80.0 kg; body condition score (BCS): 2.26 ± 0.28; milk yield (MY) in the previous lactation: 12005 ± 1384 kg; average lactation length: 177 ± 77 d (mean ± SD)] were blocked by parity, BW, BCS, MY in the previous lactation and average lactation length. They were allocated to two balanced groups (18 cows each) and moved to six separate free-stall pens (3 pens were assigned to each experimental group with 6 cows each).

All the cows were milked twice a day, at a 12-hour interval (0630 am and pm), and fed a total mixed ration (TMR), formulated in accordance with NRC (2001) protein requirements (NRC 2001) and with INRA (1989) (INRA 1989) energy requirements (Table 1).

The TMR was provided once daily at 0830. A target of 5% refusal for each pen ensured that cows had ad libitum access to feed. Representative samples of TMR, hay, corn silage and concentrate were collected weekly. Samples were pooled and analysed to assess the chemical composition of the feed. The dry matter

| Table 1. Composition and characteristics of the experimental diet fed to the 36 multiparous Italian Holstein dairy cows during the experimental period. |
|-----------------------------------------------|
| Item, % Dry Matter (DM)                       |
|-----------------------------------------------|
| Corn silage                                   | 36.38 |
| Alfalfa hay                                   | 17.44 |
| Supplementa                                   | 17.10 |
| Cornmeal                                      | 17.05 |
| Grass hay                                     | 6.12  |
| Sorghum meal                                  | 3.27  |
| Barley meal                                   | 2.64  |
| Vitamin and trace mineral supplementb         | 0.14  |
| Chemical composition, unitc                   |       |
| Dry matter, %                                 | 52.05 |
| Crude protein                                 | 15.24 |
| Ether extract                                 | 3.43  |
| aNDFOM                                        | 37.95 |
| ADFOM                                         | 20.57 |
| ADLOM                                         | 3.93  |
| Starch                                        | 27.12 |
| Crude ash                                     | 3.43  |

*Containing: Soybean meal (48% C.P.) 38.5%, Sunflower meal 18%, Corn gluten meal 18%, Soybean flakes 15%, Sodium bicarbonate 3.4%, Calcium carbonate 3.4%, Magnesium Oxide 1.2%, Dicalcium phosphate 0.8%, Sodium chloride 0.8%, Zinc sulphate 0.1%. bContaining: vitamin A (E672) 35,000 U; vitamin D3 (E571) 2641 U; vitamin E (E307) 200 mg; copper sulphate pentahydrate (E4) 93 mg; zinc oxide (E6) 238 mg; manganese oxide (E5) 50 mg; potassium iodide (E2) 4.1 mg, sodium selenium (E8) 1.3 mg; cobalt carbonate (E3) 0.57 mg. cData are expressed as % DM when no units are given. aNDFOM: organic matter from α-amylase neutral detergent fibre, ADFOM: organic matter from acid detergent fibre; ADLOM: organic matter from acid detergent lignin.
was measured by oven drying to constant weight at 65 °C. Samples were grinded with a knife mill equipped with a 1-mm sieve (Wiley model 3: Arthur H. Thomas Co., Philadelphia, PA USA). After grinding, samples were analysed for crude protein (992.23, AOAC, 2005), ash (942.05, AOAC, 2005), ether extract (920.39, AOAC, 2005), acid detergent fibre and lignin (973.18, AOAC, 2005) and starch (996.11, AOAC, 2005) using a K-TSTA assay kit (Megazyme International, Bray, Ireland). The neutral detergent fibre, amylase-treated, ash-corrected neutral detergent fibre (aNDFOM), but without addition of sodium sulphite, was determined according to Van Soest et al. (1991). Analysis results were used to calculate the nutritional values of the feed, in accordance with NRC (2001) guidelines.

Cows in the two homogeneous groups received either a control TMR without supplements (CTR) or the same TMR supplemented with 50 g/cow/day of a commercial feed additive (Al878 Valkalor®, IDENA, Sautron, France; TRT) as top-dressing. All the cows were restrained by a self-capturing headlock for about half an hour after TMR delivery and the complete consumption of the additive was monitored by visual inspection throughout the experimental period. Experimental groups were included in an 11-week crossover design consisting of two phases of 35 days each. During each phase, 14 days were used to allow cows to adapt to the experimental diet. Periodic checks were carried out between 15 and 35 days from the beginning of each phase (days from enrolment, DFE), as shown in Figure 1 and described in the following sections. At 35 DFE, the commercial feed additive administration was interrupted for a 7-days period, and CTR and TRT groups were inverted for the second phase of the experiment.

Body condition score, body weight, dry matter intake, rumination time and milk yield

On each animal, the BCS was determined by the same operator using a 1 to 4 scale (Agricultural Development and Advisory Service 1986) at 0 (before treatment administration), 14 and 35 DFE and the BW was recorded daily with an automated walk-over weighing system at the milking parlour exit (Cardinal Detecto, 102 East Daugherty St. Webb City, MO, USA). The feed intake was measured daily by weighing the amount of feed offered and refused for each pen. The daily dry matter intake (DMI) of each pen was calculated based on the dry matter concentration of the TMRs. Individual rumination time (RT) was registered using the Ruminact system (SCR Europe, Podenzano, PC, Italy), and data were processed according to Soriani et al. (2012). The MY of each animal was measured in the milking parlour by using the Afikim system (SAE Afikim, Kibbutz Afikim, Israel).

Rumen fluid collection and analysis

Rumen samples were collected on each animal with a stomach tube (Ruminator, Proofs Products, Guelph, Canada) at 35 DFE, 6 hours after the morning feed, discarding the first 500 ml of fluid collected to reduce the potential contamination with saliva. Rumen fluid samples were processed and analysed for pH, ammonia, total volatile fatty acid (VFA) concentrations and the molar proportion of acetic (C2), propionic (C3), butyric (C4), isobutyric, valeric, isovaleric, caproic and enanthic acids according to Mezzetti et al. (2019). The acetic acid to the propionic acid ratio (C2/C3) and the ratio between the sum of acetic acid and propionic acid to butyric acid [(C2 + C3)/C4] was calculated.

Milk sample collection and analysis, feed and nitrogen use efficiency calculation

Milk samples were collected on each animal by pooling milk on a weighted basis relative to milk yield from the morning and evening milking at 0 (before treatment administration), 15 and 35 DFE. Samples were assessed for composition (fat, protein, lactose, casein, urea nitrogen concentration and titratable
acidity), somatic cell count (SCC, expressed as a linear score in accordance with Wiggans and Shook (1987)) and cheese-making properties (rennet clotting time (r) and curd firmness at 30 minutes (a30)) according to Mezzetti et al. (2019). The output of fat, protein, lactose and casein and the fat to protein ratio were also calculated. The true protein value was calculated as the difference between the protein and urea nitrogen concentration expressed on a protein-equivalent basis (N x 6.38), and the energy corrected milk (ECM) was calculated according to Tyrell and Reid (1965). For each group, the feed efficiency was calculated as the ratio between the ECM and the DMI, and the nitrogen use efficiency was calculated according to Foskolos and Moorby (2018).

**Blood sample collection and analysis**

Before the morning feed, blood was collected from the jugular vein of each animal at 0 (before treatment administration), 15 and 35 DFE into 10-mL evacuated heparinised tubes (BD Vacutainer, BD Diagnostics, Franklin Lakes, New Jersey, United States). Samples were processed and assessed for packed cell volume, glucose, non-esterified fatty acids (NEFA), beta hydroxybutyrate (BHB), urea, creatinine, total bilirubin, aspartate aminotransferase (AST-GOT), gamma glutamyl transferase (GGT), total protein, globulin, haptoglobin, aspartate aminotransferase (AST-GOT), gamma glutamyl transferase (GGT), total protein, globulin, haptoglobin, albumin, cholesterol, paraoxonase, total reactive oxygen metabolites (ROMt) and ferric reducing antioxidant power (FRAP) according to Mezzetti et al. (2019). The albumin to globulin ration was also calculated to assess the inflammatory condition of the animals (Cattaneo et al. 2021). Further details of the blood analytical procedures are reported in Table S1.

**Statistical analysis**

Data were analysed using SAS software, version 9.4 (SAS Inst. Inc., Cary, NC, USA) and are presented in graphs and tables as means and pooled standard error for individual means of treatments over time. Data were analysed using a mixed model for repeated measures (Glimmix Procedure, SAS Instruments, Inc.) in accordance with Littell et al. (1998). The fixed effects of treatment (TREAT; CTR, TRT) and phase (1, 2) were considered in the model. For DMI, BW, BCS, RT, MY, feed and nitrogen utilisation efficiency, plasma and milk analytes, the fixed effect of time and the interaction effect of treatment x time (TREAT x time) were also considered in the model. For parameters determined at the pen level (DMI, feed efficiency and nitrogen use efficiency measures), the time and the pen were considered as random effects, while the time and the cow nested within the pen were considered as random effects for other parameters.

\[ y_{hijk} = \mu + \alpha_h + \beta_i + \delta_{hj} + \tau_k + (\alpha \times \tau)_hk + e_{hijk}, \]

where \( y_{hijk} \) is the response at time \( k \) on the pen or animal nested within the pen \( j \) during the phase \( i \) in TREAT group \( h \), \( \mu \) is the overall mean, \( \alpha_h \) is a fixed effect of TREAT group \( h \), \( \beta_i \) is a fixed effect of phase \( i \), \( \delta_{hj} \) is a random effect of the pen or animal nested within the pen \( j \) in TREAT group \( h \), \( \tau_k \) is a fixed effect of time \( k \), \( (\alpha \times \tau)_hk \) is a fixed interaction effect of TREAT group \( h \) with time \( k \), and \( e_{hijk} \) is random error at time \( k \) on the pen or animal nested within the pen \( j \) during phase \( i \) in TREAT group \( h \).

The analysis was carried out using three covariance structures: autoregressive order, compound symmetry and spatial power, with their heterogeneous counterparts. These were ranked according to the Akaike information criterion, with the one having the lowest value chosen (Littell et al. 1998). A preliminary analysis was conducted on DMI, BW, BCS, RT, MY, plasma and milk analytes. These were covariate-adjusted using data collected at 0 DFE, adopting \( p \leq .1 \) as a cut-off for covariate inclusion. None of the parameters had a significant covariate effect in the preliminary analysis and the covariate was therefore removed from the final model. The pairwise comparison was carried out using the least significant difference test. Post-hoc comparisons were discussed when the \( P \)-value for the main effect was 0.05 or less. The main effects at \( p \leq .1 \) are discussed in the context of tendencies.

**Results**

**Dry matter intake, milk yield, rumination time, body condition score and body weight**

The DMI and MY were higher in TRT cows compared with CTR cows \( (p = .03 \) and \( p < .01 \); Figure 2(a,b), respectively). The RT was also higher in TRT cows compared with CTR cows from 22 to 29 DFE \( (p < .05 \); Figure 2(c)). No difference between groups was detected for the BW and BCS (Figure S1(a,b)).

**Rumen fluid composition**

The rumen fluid composition is in Table 2. The rumen pH was above 6 in both experimental groups, but it was lower in TRT than CTR cows \( (p = .04 \). The total VFA concentration was higher in TRT than CTR cows.
p = .01), and the molar proportion of butyric acid tended to be lower in TRT than CTR cows (p = .09). No effect was detected for the other analytes.

**Milk quality and feed and nitrogen use efficiency**

The milk quality analysis (Table 3) showed that lactose concentration tended towards a TREAT x time interaction (p = .06). The lactose concentration was higher in the TRT cows than in the CTR cows at 35 DFE (p < .01). No effect was detected for the other analytes, or for outputs and feed and nitrogen use efficiency.

### Plasma metabolic profile

**Energy and protein metabolism biomarkers, and kidney function indicators**

Among the energy metabolism biomarkers, BHB showed a TREAT x time interaction (p < .01). The concentration of BHB was lower in the TRT cows than in the CTR cows at 15 DFE (p < .01, Figure 3(a)). No effect was seen for the PCV, or the plasma concentrations of glucose, NEFA, urea, and creatinine (Figure S2(a–e)).

**Liver function, inflammation, and oxidant status biomarkers**

Among liver function biomarkers, AST-GOT was higher in TRT than CTR cows (p < .01) and had a tendency towards a TREAT x time interaction (p = .09). The concentration of AST-GOT was higher in TRT than CTR cows at 15 DFE (p < .01, Figure 3(b)). The GGT concentration tended to be higher in TRT than CTR cows (p = .09; Figure 3(c)). Bilirubin concentration was not affected by TRT (Figure S1(f)). Among the inflammation biomarkers, no TREAT effect was seen for plasma concentrations of protein and globulin or the albumin/globulin ratio (Figure S3(a,b,d)). Among the positive acute phase proteins, haptoglobin was affected by a TREAT x time interaction. Plasma concentration...
of haptoglobin in TRT cows was lower compared to these of CTR cows at 15 DFE ($p < .05$, Figure 4(a)). Plasma concentration of ceruloplasmin was lower in TRT cows when compared to those in CTR cows ($p = .04$, Figure 4(b)). Among negative acute-phase protein biomarkers, plasma concentrations of albumin and paraoxonase were higher in TRT compared with CTR cows ($p = .01$ and $p < .01$, respectively; Figure 4(c,d)). Both albumin and paraoxonase showed a TREAT X time interaction ($p = .04$ and $p < .01$, respectively). Both plasma concentrations of albumin and paraoxonase were higher in TRT cows when compared to those in CTR cows at 15 DFE ($p < .01$). No TRT effect was found for plasma concentration of cholesterol (Figure S3(c)). Among the oxidant status biomarkers, no effect was seen for plasma concentration of ROMt and FRAP (Figure S3(e,f)).

**Discussion**

In our study, Valkalor increased MY and milk lactose concentration at 35 DFE, which is consistent with preliminary results of two previous studies conducted on mid-lactation Holstein cows (Ferguson et al. 2020; Pomport et al. 2020). Mammary lactose synthesis drives the water uptake by the mammary gland and, consequently, the milk yield (Lemosquet et al. 2009), which likely accounts for the positive effect we detected on MY but not on ECM in cows receiving the additive. As lactose synthesis primary depends on

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**Table 3.** Energy corrected milk, milk composition, feed and nitrogen use efficiency and somatic cell count in dairy cows receiving a control total mixed ration or cows receiving a total mixed ration supplied with 50 g/d of a commercial feed additive between 1 and 35 days from the enrolment.

| Item, unit | TREAT a  | Days from enrolment | p-Value | SEMb  |
|-----------|----------|---------------------|---------|-------|
| Energy corrected milk, kg | | 15 | 35 | 0.51 | 0.91 | 0.56 | 0.40 |
| Feed efficiency - | | 1.32 | 1.33 | 0.05 | 0.34 | 0.88 | 0.98 | 0.69 |
| Nitrogen use efficiency - | | 0.29 | 0.30 | 0.01 | 0.42 | 0.94 | 0.85 | 0.47 |
| Fat, g/100 mL | | 4.03 | 4.05 | 0.11 | 0.19 | 0.34 | 0.96 | 0.66 |
| Fat output, g | | 1201.60 | 1217.10 | 48.80 | 0.73 | 0.82 | 0.61 | 0.35 |
| Total protein, g/100 mL | | 3.57 | 3.64 | 0.06 | 0.10 | 0.70 | 0.49 | 0.27 |
| Protein output, g | | 1074.80 | 1099.20 | 40.90 | 0.60 | 0.75 | 0.77 | 0.26 |
| Fat/protein ratio - | | 1.12 | 1.11 | 0.02 | 0.73 | 0.26 | 0.59 | 0.78 |
| Lactose, g/100 mL | | 4.98 | 4.92 | 0.04 | <0.01 | 0.67 | 0.50 | 0.06 |
| Lactose output, g | | 1505.11 | 1511.61 | 64.30 | 0.15 | 0.70 | 0.75 | 0.54 |
| Casein, g/100 mL | | 2.68 | 2.71 | 0.04 | 0.20 | 0.74 | 0.74 | 0.33 |
| Casein output, g | | 805.73 | 820.00 | 30.3 | 0.52 | 0.74 | 0.66 | 0.28 |
| Titratable acidity, °SH/50 mL | | 3.39 | 3.37 | 0.06 | 0.34 | 0.61 | 0.40 | 0.80 |
| Urea-N, mg/100 mL | | 22.40 | 21.60 | 0.69 | 0.65 | 0.59 | 0.43 | 0.44 |
| True protein, g/100 mL | | 3.43 | 3.50 | 0.06 | 0.11 | 0.67 | 0.43 | 0.23 |
| Somatic cells count, Linear score | | 2.71 | 3.05 | 0.36 | 0.67 | 0.23 | 0.06 | 0.85 |
| Clotting time (r), min | | 18.50 | 17.70 | 0.68 | 0.73 | 0.61 | 0.61 | 0.08 |
| Curd firmness (a30), mm | | 19.20 | 17.90 | 1.35 | 0.74 | 0.99 | 0.03 | 0.55 |

aTreatment (CTR is cows receiving a control total mixed ration; n = 3 for feed and nitrogen use efficiency and n = 18 for other parameters; TRT is cows receiving a total mixed ration supplemented with 50 g/d of a commercial feed additive; n = 3 for feed and nitrogen use efficiency and n = 18 for other parameters). bStandard error = largest standard error for the fixed effects. cTreatment X time interaction (** is $p < .01$ for differences among means within a column. These symbols are only presented when the interaction effect is significant). dFeed efficiency = pen energy corrected milk / pen dry matter intake. eNitrogen use efficiency = [(pen protein output/6.38)/(pen dry matter intake * 1000 * 15.24 - 0.01 * 0.16)]. fTrue protein = {total protein - [(urea-N * 6.38)/1000]}. 

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*Table 3.* Energy corrected milk, milk composition, feed and nitrogen use efficiency and somatic cell count in dairy cows receiving a control total mixed ration or cows receiving a total mixed ration supplied with 50 g/d of a commercial feed additive between 1 and 35 days from the enrolment.
Propionate availability in ruminants, these results could be because of the higher DMI observed in TRT cows. In addition, the TRT cows had increased RT between 22 and 29 DFE. These observations, together with the higher VFA concentration we detected in the rumen liquor of TRT cows compared with those of CTR cows, suggest Valkalor administration improves feed utilisation at the level of the rumen. These results agree with those from a recent preliminary study, that found Valkalor to favour the VFA production through altering bacteria-methanogen cohorts and exerting a hydrogen-sparing effect in the rumen (Pitta D., personal communication). It is likely that HS plays a fundamental role in this respect, as recent studies found that this nutraceutical plant increases VFA production in sheep (Abdullah et al. 2020), and modulates rumen function through increasing dry matter digestibility and reducing methane emissions in dairy cows (Anele et al. 2020). A contribution of other components of the additive is also possible. In particular, eugenol, the main active component in clove bud, is known to increase the production of propionate and decrease acetate and methane in the rumen (Calsamiglia et al. 2007). Furthermore, yeast-based products contained in Valkalor are known to improve feed intake and digestibility by increasing total rumen bacteria (Newbold et al. 1998; Desnoyers et al. 2009; Patra 2012), which is likely to have a positive effect on rumen fermentation. The higher VFA production, which we found with Valkalor, agrees with the lower pH which we detected in the rumen of TRT cows compared to CTR cows suggesting a higher postprandial fermentative activity, as VFA production is one of the main factor regulating rumen pH (Penner 2010). Anyway, pH values measured in the rumen of TRT cows was in the physiological range for an optimal fibre digestion (behind 6.3 at 6 h after meal) (Dijkstra et al. 2012).

We found a higher concentration of AST-GOT and GGT in the plasma of TRT compared with CTR cows at 15 DFE. Although an acute raise in plasma concentrations of these liver enzymes is widely accepted as indicative of liver damage (Rodriguez-Jimenez et al. 2018), a moderate increase of plasma concentrations of these liver enzymes within their physiological ranges, paired with the decreased ketogenesis detected in the plasma of TRT cows at 15 DFE, most likely reflects an increased liver activity, and an overall good liver function. This suggests that the additive improved the oxidising capacity of the liver against NEFA and increases the activity of liver enzymes involved in the amino acids metabolism, likely contributing in the positive effects detected on MY. This is consistent with the reported hepatoprotective effect of HS and its ability to attenuate mitochondrial dysfunctions (Da-Costa-Rocha et al. 2014).

At 15 DFE, we found a reduced plasma concentration of α-globulins (haptoglobin and ceruloplasmin) in TRT cows. These are referred to as positive acute-phase proteins (APP) because their hepatic synthesis increases during inflammation (Ceciliani et al. 2012). The observed reduction of positive APP was concomitant with an increase in plasma concentrations of

![Figure 3](image-url)

Figure 3. Time course of plasma concentrations of beta-hydroxybutyrate (a), glutamate-oxaloacetate transaminase (GOT, b) and gamma-glutamyl transferase (GGT, c) in dairy cows receiving a control total mixed ration (CTR; n = 18; black striped bars) or cows receiving a total mixed ration supplied with 50 g/d of a commercial feed additive between 1 and 35 days from the enrolment (TRT; n = 18; green solid bars). Values are plotted as mean and SEM. ** is p < .01; + is p < .1; DFE is days from enrolment.
albumin and paraoxonase in TRT cows. These are referred to as negative APP because they decrease during inflammation due to the shift of the liver synthesis to the positive APP (Bertoni et al. 2008; Bertoni and Trevisi 2013). The APPs response seen in TRT cows suggest Valkalor to be effective in mitigating the acute phase response at the liver level. This is consistent with the anti-inflammatory effect exerted by flavonoids, polysaccharides and organic acids contained in HS through inhibiting the production of proinflammatory cytokines (i.e. interleukins, interferons and tumour necrosis factor alpha) (Dafallah and Al-Mustafa 1996; Reanmongkol and Itharat 2007). The augmented rumen fermentation recorded in the TRT group could also have reduced the fermentation of feedstuffs in the hindgut, which in turn may have mitigated the inflammatory condition of the animals. Excessive hindgut fermentation is known as a factor that stimulates the acute phase response in ruminants (Minuti et al. 2014). An anti-inflammatory effect of Valkalor could further account for the increased liver activity in our TRT cows. The occurrence of an acute phase response is known to perturb normal liver functions and to inhibit enzyme efficiency (Trevisi et al. 2012; Shahzad et al. 2014). Furthermore, the contribution of the anti-inflammatory activity of HS resulting in the higher DMI and RT we observed in TRT cows could be also hypothesised. Proinflammatory cytokines have an anorexic effect in dairy cows through reducing feed intake (Kuhla 2020), and RT is known as a reliable marker of such an impaired feed intake (Calamari et al. 2014).

Although these results suggest a positive effect of the additive on dairy cows metabolism and performance, likely driven by the combined action of its active compounds on the rumen fermentations and liver metabolism, we have to point out that this study has two potential limitations: i) the patent covering the commercial formula of the feed additive used in this study (Al878 Valkalor ®) does not allow us to clearly identify the mode of action of the active components included in it, and ii) the facilities used to perform this study did not allow us to measure individual DMI of the animals, therefore group measurements were used. Thus, results obtained in this study should be verified in experimental conditions where individual feed intake measurement will be possible.

**Conclusions**

In our study, the higher DMI and RT observed, together with the improved rumen fermentation of
the TRT cows, compared with CTR cows, suggest Valkalor administration improves feed utilisation. This may be a result of the positive effects of the combination of yeast-based products, clove, and coriander essential oils and HS contained in the feed additive on the fibre-fermenting bacteria at rumen level. The positive effects on feed intake and feed utilisation are likely to account for the increased MY and milk lactose concentration of our TRT cows. The positive effects of the additive on reducing ketogenesis, mitigating the acute phase response, and increasing liver activity suggest a probable hepatoprotective and anti-inflammatory effect of the product. These effects could arise from the increased energy availability resulting from the positive effects of the additive seen at the rumen level, and on its effectiveness in mitigating hindgut fermentation. The positive effects of Valkalor on mid-lactation cows seen in this study suggest that this feed additive could be of value for transition dairy cows, that are usually in negative energy balance and therefore may be more responsive to the positive effects on digestion. Valkalor may also protect against the severe dysfunctions occurring in the liver, in the energy metabolism, and in the inflammatory response of transition dairy cows. Therefore, a more in-depth study of its mode of action at the metabolic level would be useful. In addition, the persistence of such effects should be further investigated, as we observed that some effects were more evident in the first weeks of diet supplementation.

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Ethical approval

This study complied with Italian laws on animal experimentation (DL n.26, 04/03/2014) and ethics (authorization of the Italian Ministry of Health N 158/2015-PR).

Disclosure statement

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Data availability statement

The data presented in this study are available on request from the corresponding author.

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