Effect of pre-treated almond by-products at different doses of fibrolytic enzyme on nutrition value, ruminal fermentation and enteric methane emission

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Effect of pre-treated almond by-products at different doses of fibrolytic enzyme on nutrition value, ruminal fermentation and enteric methane emission

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

Abstract

Almond's by-product is available in large quantities all over the world. However, little information is available about their feed value. The aim of this research was to determine their nutritional value and investigate the effect of pre-treating with exogenous fibrolytic enzymes (EFE) at 0, 1, 2, and 4 µL/ g DM.

The result showed that the almond shell is a fibrous by-product with low energy value. The pre-treatment with high doses of EFE hydrolyses part of their cellulose and hemicellulose, improved their fermentation, digestibility and energy value. In addition, it improved protein produced by the ruminal microbiota and volatile fatty acids. This improvement is coupled with the reduction of methane emission. Almond teguments are rich in fibre and have high energy value due to their high fat content. A high content of condensed tannins has been detected in this by-product. The pre-treatments with EFE did not modify their fibre composition and nutritional value. Almond hulls have a high energy value due to their high non-fiber-carbohydrates content. It has moderate fibre compounds and high condensed tannins. The pre-treatment with EFE has no effect on their fibre composition. However, at the low dose, it improves their fermentation, digestion and protein produced by ruminal microbes and at the high dose it has an unharmful effect.

This study can encourage farmers to use almond by-product as an alternative feed for ruminants. EFE is good strategy to improve nutritional value of almond by-product, reduce energy loss and protect the environment against global warming

Keywords

almond by-products, exogenous fibrolytic enzyme, nutrition value, enteric methane emission

Introduction

The current challenges in animal farming are to intensify animal production by improving feed quality and seeking alternative resources that should not compete with human nutrition. The use of agricultural by-products is becoming a current trend that can be a solution for this challenge (Lucio et al. 2021).

Almond tree (Prunus dulcis) is cultivated all over the world and mostly in the United States and Mediterranean countries. The worldwide almond fruit production is estimated at about three million tonnes per year with a very rapid growth rate in recent years (about 5% annually) (Moradi Yeganeh et al. 2021).

The blanched almond kernels (without teguments) are widely used for many foods such as almond cake, almond flour, and rice preparations.... The separation of kernels from the fruit and separation of teguments from the kernels, generates several types of by-products (hull, shell and teguments) present approximately 89% of fruit weight which are not usable in human nutrition (Garcia-Perez et al. 2021). Few data are available about the possibility of incorporating almonds by product in the ruminant's nutrition. According to Swanson et al. (2021) almond hull could be added safely to 20% in dairy cow diets as a concentrated supplement without affecting milk production or feed intake. Rad et al. (2016) found that almond hulls treated with 5% of urea could be incorporated safely to 40% in growing lambs' diets as roughage replacement (alfalfa hay) without affecting their average daily weight gain, and rumen fermentation parameter and characteristics. Can et al. (2007) reported that almond hull and shell could be used safely to 40% in goat' diets as roughage replacement (wheat straw) without affecting the digestibility and palatability of the diet. However, no information on incorporating almond teguments in the ruminant's feed.

For a better valorisation of by-products in ruminant nutrition, nutritionists' have proposed various treatments to improve their fermentation and degradability such as biological treatment based on enzymes additive. These feed additives used in animal diets, produced by safe microbial strains, catalysts accelerating the rate of the chemical reaction. The pre-treatment of agricultural by-products with EFE is becoming a current trend in animal nutrition (Zhong et al. 2021). The use of EFE in ruminants' nutrition is a safe, inexpensive and profitable method (Refat et al. 2018; Abid et al. 2020). These feed additives modify chemical composition of feed before use by animals and ruminal microbiota in the rumen (Newbold et al.1992; Abid et al 2022a). Despite the benefits offered by EFE in animal nutrition, the information available on the effect of these additives on the emission of methane gas is limited and variable. Some studies show a reduction (Beauchemin et al. 2020), others show no effect (Wang et al. 2016) and others note an increase (Chung et al. 2012).

Therefore, this research was conducted to determine the nutritional value of almonds by-products and investigate the effect of increased dose of EFE on their fibre compound, ruminal fermentation, degradability, energy value and enteric methane emission.

Materials and methods

Collect and treatment of almond by-products

Samples of fresh almond hall, almond shells and almond teguments collected from almond trees (*Acheek variety*) in Sfax region (south of Tunisia). 12 hours before the *in vitro* fermentation, almond by-products were treated with mixture 1:1 (v/v) of Cellulase plus and Xylanase plus (Dyadic International Inc. Jupiter Florida, USA) produced from T. *longibrachiatum* at four doses: 0 (control), 1 (low), 2 (medium), and 4 (high) µL/ g DM at 26°C.

Xylanase, endoglucanase and exoglycanase activities of EFE were measured at conditions similar to the rumen environment (pH 6.6 and temperature 39 °C) according to the protocols of Wood and Bhat (1988) and Biely and Poutanen 1992). This biological additive has 2267 units of xylanases/ ml, 1161 units of endoglucanase/ ml, and 113 units of exoglucanase/ ml.

Chemical analysis

The chemical composition of almond by-product, dry matter (DM) crude protein (CP), ether extract (EE), and ash was determined according to AOAC (1995) protocols. Neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) were analyzed according the protocol of Van Soest et al. (1991) by using a fibre analyzer (ANKOM220, ANKOM Technology, Macedon, NY). Hemicellulose and cellulose were calculated from the difference between NDF and ADF and between ADF and ADL respectively (Van Soest et al. 1991. Condensed tannins were analysed by using acid-butanol-HCl-Fe protocol with spectrophotometry at 550 nm of absorbance (Makkar et al. 1993)

Non fiber carbohydrates (NFC) were esteemed with equation 1:

$\mathrm{NFC} = 100 - (\mathrm{NDF} + \mathrm{CP} + \mathrm{EE} + \mathrm{NDF} + \mathrm{Ash})$ (1) (NRC 2001)

2.1. In *vitro* incubation

The *in vitro* ruminal incubation technique was realised with a syringe method flowing the protocol of Menke and Steingass (1988). The fresh rumen fluid content was obtained from 2 cows (weighted 650± 20 kg, aged 9± 1 years). Each cow is fed twice daily at a total ration composed of 7 kg DM oat hay and 3 kg DM of commercial concentrate. Before the morning feed, rumen fluid was collected. Rumen fluid filtered through four layers of cheesecloth into thermos flasks at 39°C. Inoculum of these two cows was mixed 1:1 (V/V) and immediately transported to the laboratory. The filtered ruminal fluid combined with Menke and Steingass (1988) buffered mineral solution in the ratio 1:2 (v/v) at conditions similar to rumen (anaerobic and temperature 39°C).

Samples of 0.2 g of ground almonds byproducts untreated and pre-treated with the appropriate dose of EFE were incubated with 30 ml of the buffered ruminal solution in glass syringes of 100 ml. Additionally, negatives' control syringes (containing only 30 ml of the buffered ruminal fluid) were prepared to correct gas production from the buffered ruminal fluid. Each treatment was realized in triplicate (3 reputation). These syringas were immediately incubated in a water bath at 39°C.

Gas production was measured at 2, 4, 6, 8, 12, 24, 48, 72, and 96 hours. This experience was repeated three times (3 runs). The gas produced was corrected by the gas produced in negatives' controls syringes. The kinetic of gas production of fermentation was fitting by using the nonlinear option of SAS (Version 9.3) according to Equation (2):

$GP_{(t)} = B\,(1 - e^{(-C\,t - Lag)})$ (France et al.2000)

where: GP is the gas produced at the time t in ml/g dry matter; t is the incubation time in h; B is the asymptotic gas production in ml/g dry matter; C is the constant rate of gas production in ml/ h; and Lag is delay at the start of gas production in h.

The metabolizable energy (ME), net energy lactation (NE_L) and total volatile fatty acids (VFA were calculated with Equations 3, 4 and 5:

$ME = 2.2 + 0.136 \times GP + 0.0057 \times CP$ (3) (Menke and Steingass 1988)

$NEL = 0.101 \times GP + 0.051 \times CP + 0.112 \times EE$ (4) (Menke and Steingass 1988)

$VFA = -0.00425 + 0.0222 \times GP$ (5) (Getachew et al. 1998)

ME is metabolizable energy value in MJ/ kg dry matter; NE_L is net energy lactation in MJ/ kg dry matter; VFA is the ruminal total volatile fatty acids in mmol/ 200 mg dry matter; GP is the net gas production (ml)/ 200 mg dry matter after 24 hours of incubation; CP is crude protein in g /100 g dry matter; and EE is ether extracts in g/100 g dry matter.

At the end of fermentation, methane emission was analyzed based on the protocol of Fievez et al. (2005). The contents of each serum syringe was filtered by filter paper (Whatman 541). Residual was collected and dried at 55°C for 48 to determined the dry matter digestibility (DMD) with equation 6

$$DMD = \frac{\text{initiale DM} - \text{residual DM}}{\text{initiale DM}} \times 100 \quad (6)$$

Microbial crude protein was determined with equation 7

$MCP = DMD - 2.2 \times GP$ (7) (Blümmel et al. 1997)

Where MCP is microbial crude protein in mg/ g dry matter; DMD is the amount of dry matter digestibility in mg/ g at the end of incubation; GP is the net gas production (ml)/ 200 mg of DM of substrate at 24 hours of fermentation, on, and the 2.2 mg/ ml is a stoichiometric factor.

Statistical analysis

All data were analysed by using as a 4 × 3 factorial arrangement 4 doses of EFE (0, 1, 2, and 4) and 3 almonds by product (almond hull, almond shell, and almond teguments) in completely randomised design by using the general linear model procedure of SAS 9.3 flowing equation. (8):

$$Y_{ijk} = \mu + D_i + S_j + \varepsilon_{ijk} \quad (8)$$

Where: Y_{ijk} present the observation; μ present the overall mean; D_i present the effect of the ith dose (i = control, low, medium, and high), S_j present the effect of jth substrate (j= almond hull, almond shell and almond tegument), and ε_{ijk} present the random residual error.

The orthogonal contrasts were used to examine the linear and quadratic effects of doses for each by-product. Duncan's multiple range tests were performed to separate means and significance was declared when p <0.05.

Result

The chemical composition of almond by-products is presented in table 1. Almond shells have huge fibre contents. Almond teguments have huge fibre contents, fat content and condensed tannin. Almond hull has huge non-fiber-carbohydrates and condensed tannin, and moderate fibre compound.

Insert TABLE 1

The effect of EFE in fibre compounds is illustrated in figure 1. EFE decreases cellulose and hemicellulose compounds of almonds shell at high dose and does not affect fibre compounds of almond hull and almond teguments.

Insert FIGURE 1

The parameters of fermentation, and nutritional value of almonds by product (untreated or treated with EFE) are shown in Table 2. Almond shell is characterized by low fermentation, dry matter digestibility, and energy value. Adding EFE increased their asymptotic gas production from 118.3 to 139.1 ml/ g DM and constant rate of gas production from 0.044 to 0.054 ml/ h and decreased the lag time from 2.11 to 1.17 h. This modification of fermentation coupled with an increase of DMD by 8.2 g/100 g, ME by 0.47 MJ/ Kg DM, EN_L by 0.35 MJ/ Kg DM, VFA by 0.08 mmol/200 mg DM and MCP by 81.5 mg/ g dry matter. Also, at this dose it decreases methane emission by 2.5 ml/ g DM.

Almond teguments and almond hull characterised by moderate digestibility and energy value. Pre-traded almond teguments with EFE do not affect their nutrition value. For almond hulls, EFE at low dose improves their fermentation asymptotic and digestibility. However, the high dose has unharmful effect

Insert TABLE 2

Discussion

Almond hull is characterized by moderate fiber compounds (NDF= 25.1 g/100 g DM). This data was in agreement with the results of Swanson et al. (2021) (NDF= 23.8 g/100 g DM). However, almond shells and teguments have high fiber compounds 92.2 and 51.8 g/100 g DM respectively. These results are higher than the previous data found by Saura-Calixto et al. (1983) in almond teguments (NDF=46.43 g/100 g DM) and in almond shells (NDF= 90.31 g/100 g DM). This difference may be due to the varietal diversity of almonds. Based on fiber compounds and according to recommendation of NRC (2001) almond shell and teguments can replace classic forage without disturbing the normal functioning of the rumen. Almonds hull can be used as a fibrous ingredient in ruminant concentrates or at low fiber fodder associated with fiber fodder to avoid the risk of ruminal acidosis. It should be noted that the undegradable fiber fraction (ADL) of these feeds is very high 8.3, 14.1, 23.6 g/100 g DM for almond hull, almond teguments and almond shell respectively.

These results in almond hull and almond shell are lower than the previous data found Saura-Calixto et al. (1983) in almond hull (10.01 g/100 g DM) and almond shell (27.8 g/100 g DM) and higher than the previous data found Saura-Calixto et al. (1983) In almond tegument (7.5 g/100 g DM). This indigestible compound blocks the attachment of the microorganism to the substrate, and protects the other feed component to hydrolysis enzymes produced by ruminal bacteria (Zhong et al. 2021). Therefore, these feeds must be treated to reduce this compound or/and add additives to facilitate the activity of the ruminal microbiota. The crude protein content in the almond shell and hull (0.8 to 2.3 g/100 g DM) is very low. These data are similar to those reported Saura-Calixto et al. (1983) (1 to 2.7 g/100 g DM). It is lower than the CP of wheat straw (4.3 g/100 g DM) (Abaş et al. 2005). The use of these by-products in ruminant feed does not cover the protein maintenance needs of ruminants (6–8 g/100 g DM) (Van Soest et al. 1994). Therefore, it is recommended to be combined with foods rich in protein.). The CP of almond tegument (7.6 g/100 g DM) can cover the CP need of maintenance of ruminants (Van Soest et al. 1994). These data are lower to those reported by Saura-Calixto et al. (1983) (10.5 g/100 g DM). In addition, it is very interesting to note that almond hulls and almond tegument are characterized by high content in condensed tannin (3.8 to 5.1 g/100 g DM). This secondary metabolite improves oxidative stability, sensory quality of meat and milk, reduces methane emission and improves animal health and increases the absorption of amino acids in the small intestine. However, it has a depressive effect in the palatability, ruminal digestion of protein and fiber, microorganism growth and meat and milk production (Vasta et al. 2019; Kelln et al. 2020; Serra et al. 2021). Moreover, almonds hull contain a highly fermentable carbohydrate (NFC=62.8 g/100 g DM). This data is comparable to those reported Swanson et al. 2021 (NFC=64 g/100 g DM). It is similar to NFC contents in barley grain (NFC= 63.96 g/100 g DM) (Heydari et al. 2021). According to Offeman et al. (2014) the carbohydrate of the almond hull is composed essentially by sucrose, fructose, glucose, inositol and sorbitol. Almond shell and almond tegument has very low NFC (5.2 g/100 g DM and 17 g/100 g DM) which is lower than that of the content found corn silage (28.77 g/100 g) (Heydari et al. 2021). Moreover, almond teguments have high fat content (EE= 17.3 g/100 g DM). This data is higher than the result found by Saura-Calixto et al. (1983) (14.3 g/100 g DM). It is lower than fat contents in rice bran (EE=22 g/100 g DM) (Rafe et al. 2007 . This energy compound can block the attachment of ruminal microbiota to carbohydrate, reducing their digestion and the proliferation of ruminal microbiota (Joy et al. 2021). Almonds shell and hull have a low-fat content 0.6 and 1 g/100 g DM respectively. These findings align with a previous study conducted by Saura-Calixto et al. (1983) and similar to fat contents in wheat straw (Abaş et al. 2005).

The *in vitro* ruminal fermentation is the most common technique used for determining feed quality for ruminants (He et al. 2015). Based on their energy value (NEL and ME) almond shell has low energy value (ME= 5.35 MJ/kg DM and NEL = 2.33 MJ/kg DM) which is similar to wheat straws (ME= 5.26 MJ/kg DM and NEL = 2.67 MJ/kg DM) (Abaş et al. 2005). The teguments of almonds and almonds hull have a better energy value than the almond shell. The EM of almond is the best (ME= 8.35 MJ/kg DM) which is similar to the ME of alfalfa Hay (ME= 8.88 MJ/kg DM). The almond teguments have the best ENL(6.23 MJ/kg DM) which is similar to ENL of wheat bran (ENL = 6.38 MJ/kg DM). (Abaş et al. 2005)

The effectiveness of EFE additives depends on the substrate, and dose of EFE,. This data was in agreement with the results found by Abid et al. (2022b). The pretreatment of almond shell by EFE at high dose hydrolyzes their cell wall polymers (cellulose from 36.8 to 31.3 g/100 g DM and hemicellulose from 32.1 to 27.6 g/100 g DM). Similar effect has been proven by Abid et al. (2022b) during pre-treatment of olive cake. EFE improved the amount of fermentation and coupled with an improvement of DMD by 8.2 g/100 g DM indicate that EFE offers more available substrate for ruminal microorganisms. This increase of bioavailability of nutrients may be explained by the hydrolyze and destroy the complex structure of the fibre matrix and then improve its accessibility and even reduce certain anti-nutritional factors (Lucio et al. 2021). Therefore, this additive has improved the energy value of this feed (ME by 0.5 MJ/ Kg DM and NEL by 0.35 MJ/ Kg DM). This result is in agreement with that found by Abid et al. (2022b) in olive cake. Also, this additive reduces the delay time at the start of gas production. This effect may be due to the increase of sugars in the substrate due to hydrolyze of cellulose and hemicellulose (Abid et al. 2022b) which can act as chemoattractant for ruminal microorganisms, and therefore reduce lag time (Lopez 2005). Also, this additive increases the rate of fermentation by 0.01 ml/ h. This improvement of fermentation may be due to the increased microbiota in the rumen Newbold (1992). This improvement can increase the transit of this fibrous feed in the rumen and consequently increase the animal's ability to ingest it. This result is similar to the results shown by Abid et al (2020, 2022b) during the treatment of olive cake *in vitro* and *in vivo*.

This improvement coupled by the reduction of energy loss in the form of methane emission. Similar effect was found by Giraldo et al. (2008). Additionally, this treatment improves the protein produced by the ruminal microbe by 78.5mg/ g DM. This improvement is higher than the improvement found by Abid et al. (2022a) in brewer's spent grain (35.2 mg/ g DM) and lower than the improvement found Abid et al. (2022b) in olive leaves (83 mg/ g DM). This effect may be due to the increase in the ruminal microbiota and the synchronization between the energy and protein resources of this pre-treated feed. Also, this feed additive improves the VFA from 0.51 to 0.59 mmol per 200 mg dry matter. Similarly, Abid et al. (2022b) found improvement in VFA olive leaves from 0.57 to 0.68 mmol per 200 mg dry matter.

The pre-treatment of almond hull, with fibrolytic enzymes, at low dose not modified their fibre composition and improves their amount of fermentation and protein produced by microbiota ruminal. These improvements agree with those found by Miller et al. (2008) in low fibre feed wheat grains (NDF= 14.8 g/100 g DM). This modification may be due to synergies between the EFE and the ruminal microbiota (Newbold et al.1992). Contrary to the result found on almond shells, the high dose of EFE reduced the fermentation of almond hull and the production of protein by ruminal microbiota. The harmful effect of additives at high doses has also been proven *in vivo* (Kung Jr et al. 2000) and *in vitro* (Abid et al. 2022a; b). several hypotheses which can explain this result such as competition between endogenous and exogenous enzymes for the binding sites, or/ and competition between exogenous enzymes and the ruminal microbiota or a release of anti-nutritional elements like condensed tannin from the treated in the ruminal environment (Nsereko et al. 2000; Kelln et al. 2020).

For almond teguments, EFE does not produce any effect. The absence effect on almond teguments may be due to their high fat which can block the binding attachment of ruminal microbiota and enzyme to carbohydrate of feed and the proliferation of ruminal microbiota (Joy et al. 2021).

Conclusion

Based on this research, it could be concluded that almond by-products can be used as alternative feed for ruminants. Almond teguments can be used as energy fiber feed, almond hull can be used as energy moderate fiber feed and almond shell can be used as fiber feed. The pretreatment of almond byproducts by EFE is a good strategy to improve the nutritional value of almond shell and hull.

Data availability

All mean data are presented in the tables. The individual data may be obtained from the corresponding author with a reasonable request.

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Contributions

KA, AM, JR and KM conceived and designed research. KA, JJ, and HY conducted experiments. KA and JJ analyzed data. KA wrote the manuscript. All authors read and approved the manuscript.

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Ethics declarations

Consent to participate

All the authors were aware regarding participation and publications.

Consent for publication

All the authors were aware regarding participation and publications.

Conflict of interest

The authors declare no competing interests.

Figures

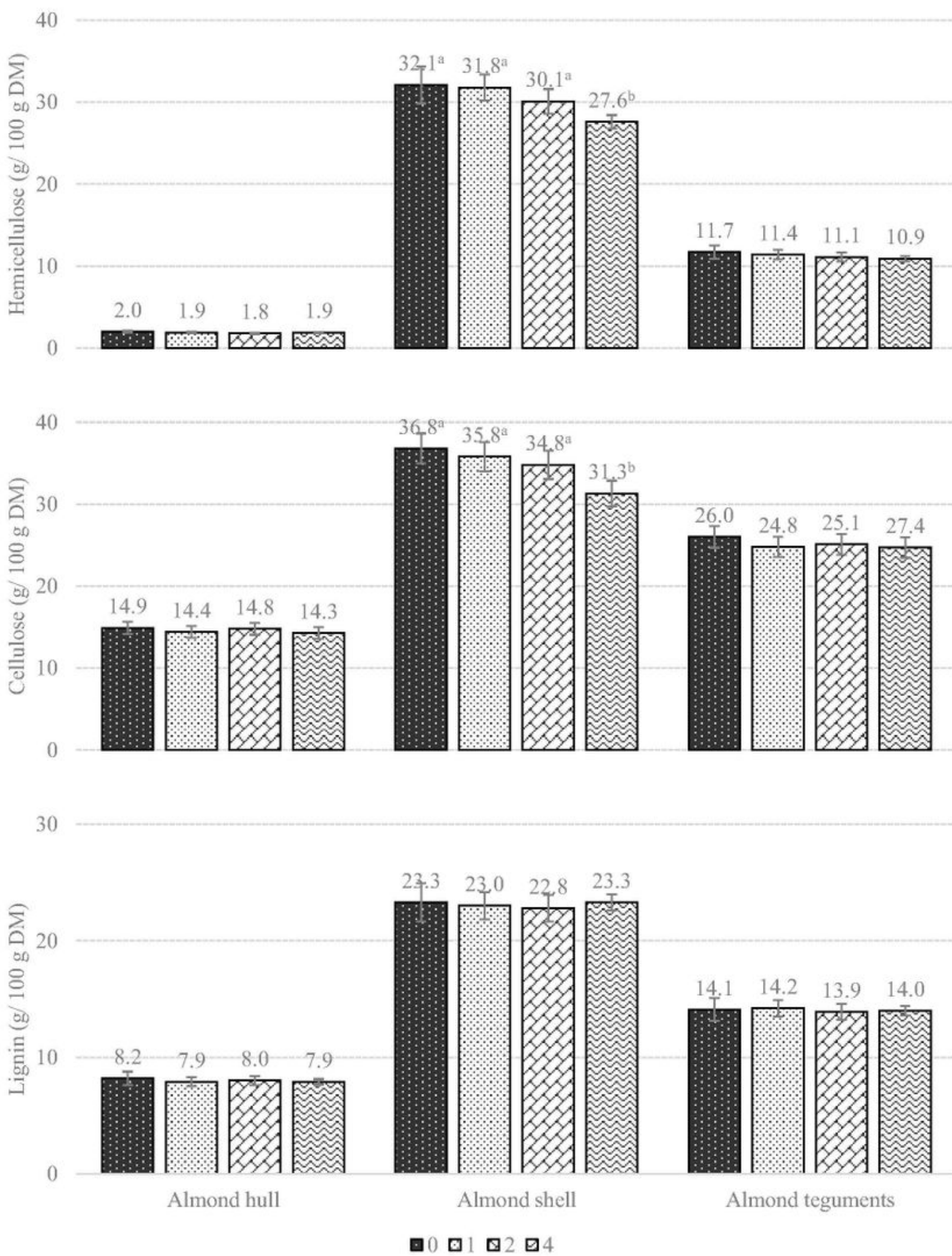

Figure 1

Effect of exogenous fibrolytic enzymes on fibre compound of almond by-products [a,b] different letter flowing the mean value of each almond by-products indicate that they are different ($p < 0.05$)

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