

Title	1
I would suggest to reword the title in a more appropriate manner	2
Answer: The title was changed to “Effects of vegetal extracts towards chickens gastrointestinal contractility: a possible way to improve farm animals life quality and health”	3 4
Abstract	5
L16 “They are mixtures of polyphenolic compounds”, the term phytocomplex could fit better	6
Answer: L16 became L19. The term phytocomplex replaced the sentence ““They are mixtures of polyphenolic compounds” as requested	7 8
L21 The results show that the chemical composition of the two phytocomplexes in-21 fluences the spontaneous intestinal contractility in different ways by regulating the tone and consequent progression of the food bolus.	9 10
How can you ascribe the function to the chemical composition?	11
Answer: L21 became L24. Basing on previous results, we speculate the tannins are the main responsible for the effects on contractility, as we demonstrated ENC, rich in hydrolysable tannins, exert antimuscarinic, antihistamic, calcium antagonistic, activities in guinea pig ileum and colon. [Budriesi R, Ioan P, Micucci M, Micucci E, Limongelli V, Chiarini A. Stop Fitán: antispasmodic effect of natural extract of chestnut wood in guinea pig ileum and proximal colon smooth muscle. J Med Food. 2010 Oct;13(5):1104-10] Also condensed tannins were shown to modify gut contractility	12 13 14 15 16 17
L24 “an important target of the effects of tannins that contributes to 24 animal and indirectly to human health.”	18
How can you be so sure that this could have some effect on human health? Please reformulate the sentence.	19
The effects on contractility may lead to a reduction of liquid faeces that may result in a decrease of orofecal transmission of pathogenic microorganisms. Therefore, the studied phytocomplexes may be used in order to reduce antibiotics in farm animals, with a consequent reduction of selection of multidrug-resistant microbiological strains. Furthermore, these extracts are rich in antioxidant compounds that may provide a meat of better quality. Our final aim will be the identification of nutraceutical with long term salutogenic effects.	20 21 22 23 24 25
The sentence “In conclusion, we can hypothesize that tannins exert an interesting effect on contractility, an important target of the effects of tannins that contributes to animal and indirectly to human health.” was changed to “The chemical analysis of the two extracts revealed that ENC mainly contains hydrolysable tannins while Q mainly has condensed tannins. The two phytocomplexes show different effects towards gastrointestinal smooth muscle contractility, with ENC providing a better activity profile than Q.”	26 27 28 29 30
Keywords: the first two are too long. Put , or ; between each keyword.	31
We corrected as requested	32
Introduction	33

The structure of the introduction is quite confused. The authors talk widely about the positive effect of chestnut extract (especially the antimicrobial) but barely mention or discuss the choice to use the quebracho one. Moreover, the authors don't go straight to the point.

Answer:

we explained the reasons why we used quebracho, adding the following sentence:

"Quebracho extract was used as it contains high amounts of condensed tannins, endowed with many biological beneficial effects. In addition, it was chosen in order to make a comparison between condensed and hydrolysable tannins, in order to understand which molecules may be further studied for applications in animals farming." in lines 81-85

In order to better explain our aim , we added the sentence "

"...and were chemically characterized, in order to investigate their possible application in the field of industrial farms." in lines 105-106

L 37 Move the cite at the end of the sentence. There is a full stop in the middle of the sentence. Please put it in a correct form.

Answer: L37 became L43.

The whole sentence was changed to:

"Therefore, the identification of nutaceuticals endowed with antimicrobial activities and with other effects, such as antiinflammatory, antioxidant and gut modulatory properties may lead to an improvement of general health status of animals and to a decrease of antibiotics need, that may result in a reduction of antimicrobial resistance. [2]"

L60 in human diseased. Correct the sentence.

Answer: L60 became L71. "human diseased" was changed to "human diseases"

L67 Why are the authors constantly talking about the antibiotic properties of tannins when the aim of the study is to assess the effect on intestine contractility?

Answer: L 67 became L 85

We speculate that the effects on the reduction of pathogenic bacteria transmission may result from the formation of more solid stools and not from a direct antimicrobial effect, that, however, was reported for many molecules found in the extracts.

L74 And should be in small caps

Answer: L 74 became L 92

"And" was changed to "and".

Results

The authors report some data about the chemical composition, but it seems that these are just citation from other works. In this case the presented data are not results of the present study and should not be included in this section. Maybe this part it could be included in the introduction and cited in the M&M section.

Answer: the results were reported because chemical composition was elucidated using the methods reported in the cited works and they were analysed also in our experiments. We added some pieces of information regarding the gravimetric analyses.

Table 2 No caption is present.

Answer: The following caption "Tannins and polyphenolic compounds concentration of ENC, and Q." was written, as requested

Figures in the panels should be marked by letters.

Figures were replaced by the following figures, following referees' statment

Figure 3

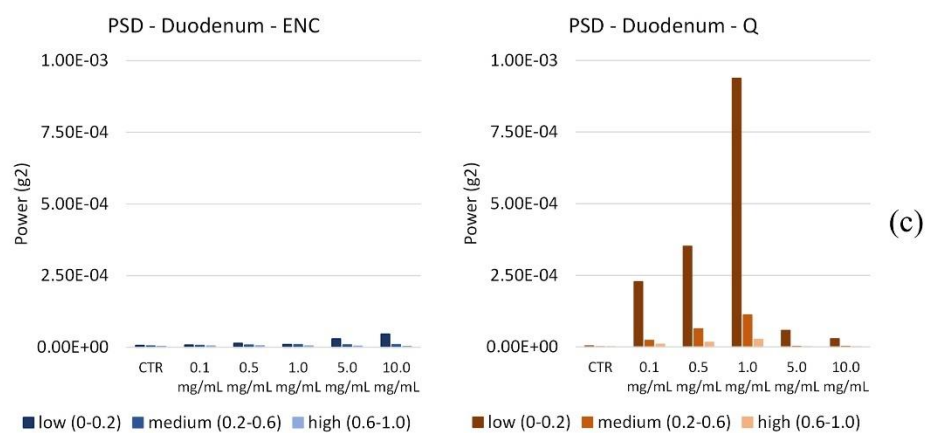
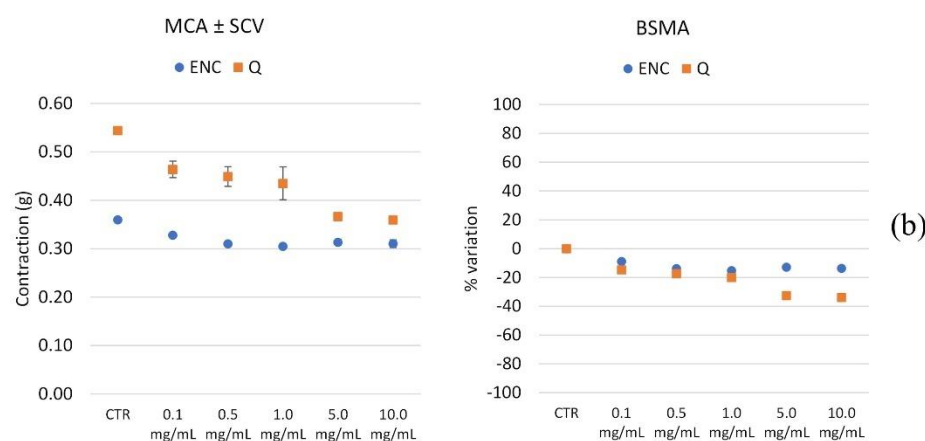
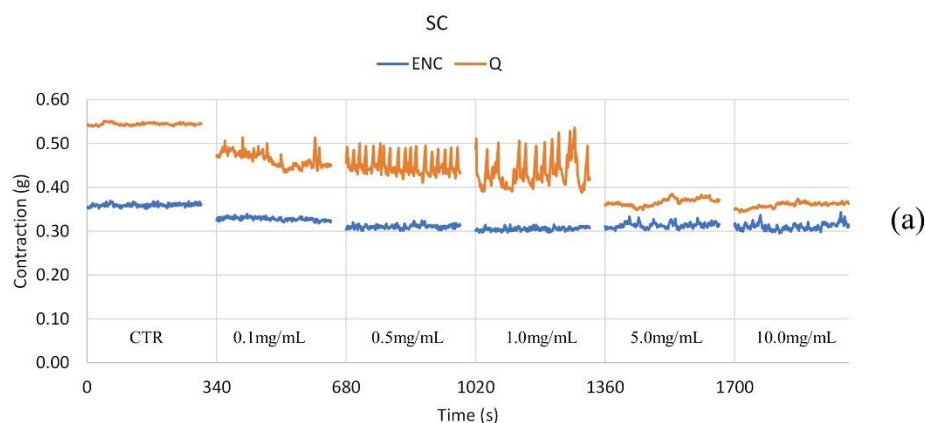


Figure 3. (a) Experimental original recording of the concentration-response curve of ENC (*Castanea sativa* Mill.), and Q (*Schinopsis balansae* Engl.) extract on duodenum spontaneous contractility (SC). (b) Mean contraction amplitude (MCA) with spontaneous contraction variability (SCV). Basal Spontaneous Motor Activity (BSMA). Zero represents the basal tone and each point is the percent variation from the baseline after cumulative addition of each dose; each value is the mean $\pm$ SEM; when the error bar is not shown, it is covered by the point. (c) Power Spectral Density (PSD).

Figure 4

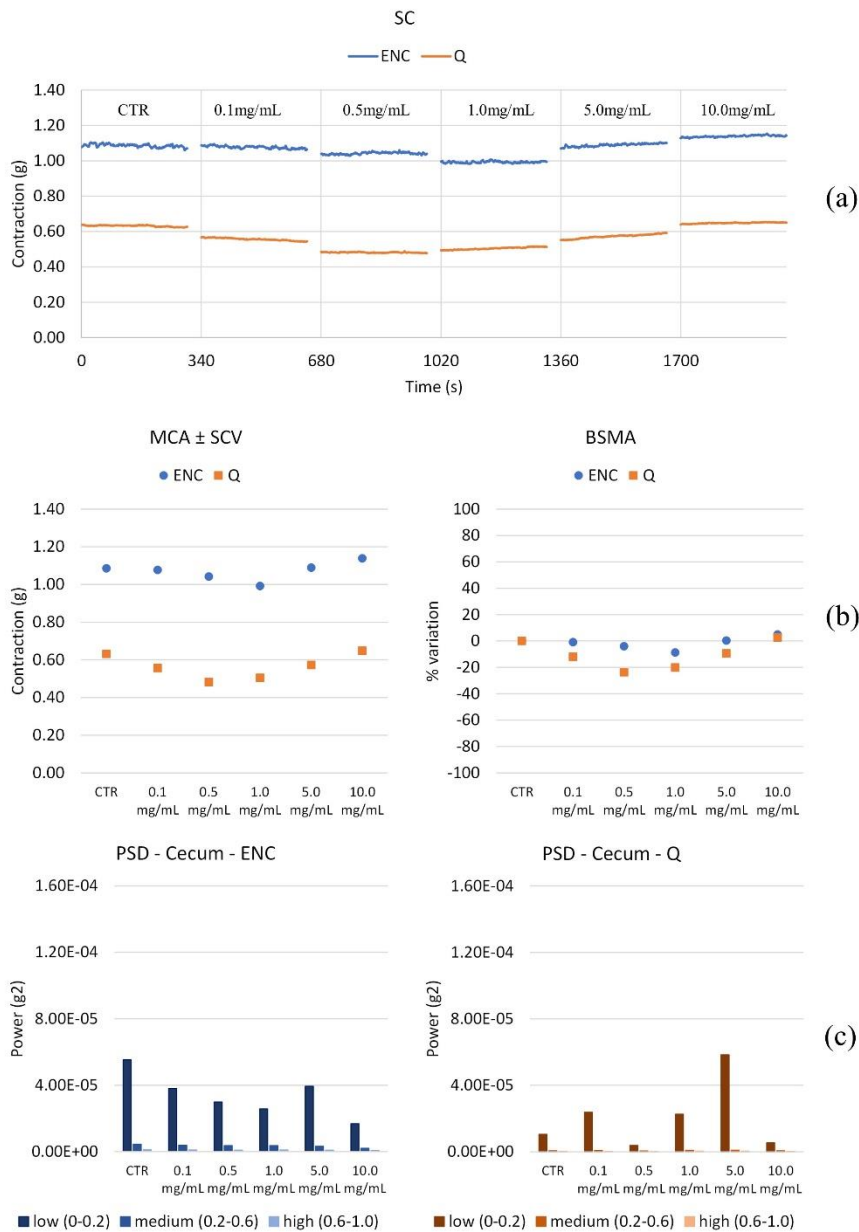


Figure 4. (a) Experimental original recording of the concentration-response curve of ENC (*Castanea sativa* Mill.), and Q (*Schinopsis balansae* Engl.) extract on caecum spontaneous contractility (SC). **(b)** Mean contraction amplitude (MCA) with spontaneous contraction variability (SCV). Basal Spontaneous Motor Activity (BSMA). Zero represents the basal tone and each point is the percent variation from the baseline after cumulative addition of each dose; each value is the mean $\pm$ SEM; when the error bar is not shown, it is covered by the point. **(c)** Power Spectral Density (PSD).

Figure 5

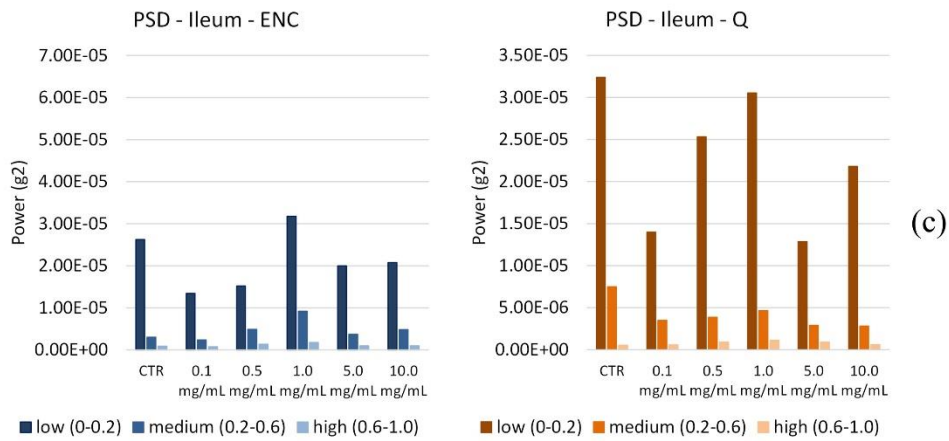
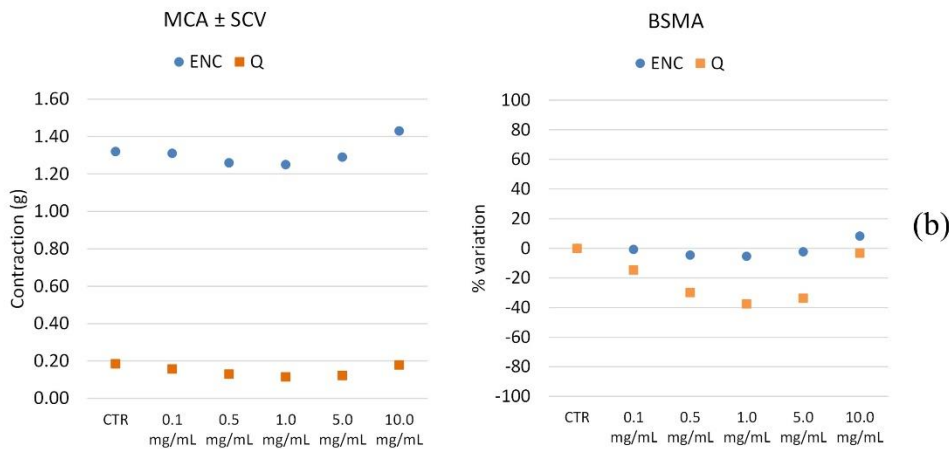
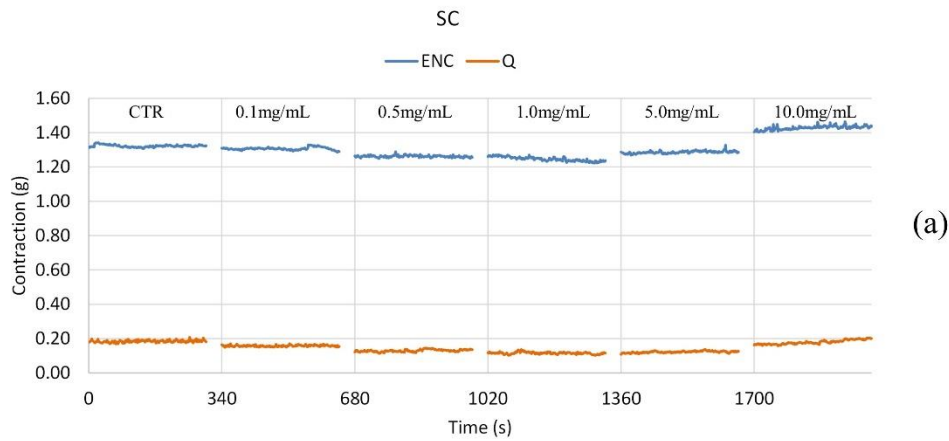


Figure 5. (a) Experimental original recording of the concentration-response curve of ENC (*Castanea sativa* Mill.), and Q (*Schinopsis balansae* Engl.) extract on ileum spontaneous contractility (SC). **(b)** Mean contraction amplitude (MCA) with spontaneous contraction variability (SCV). Basal Spontaneous Motor Activity (BSMA). Zero represents the basal tone and each point is the percent variation from the baseline after cumulative addition of each dose; each value is the mean $\pm$ SEM; when the error bar is not shown, it is covered by the point. **(c)** Power Spectral Density (PSD).

Figure 6

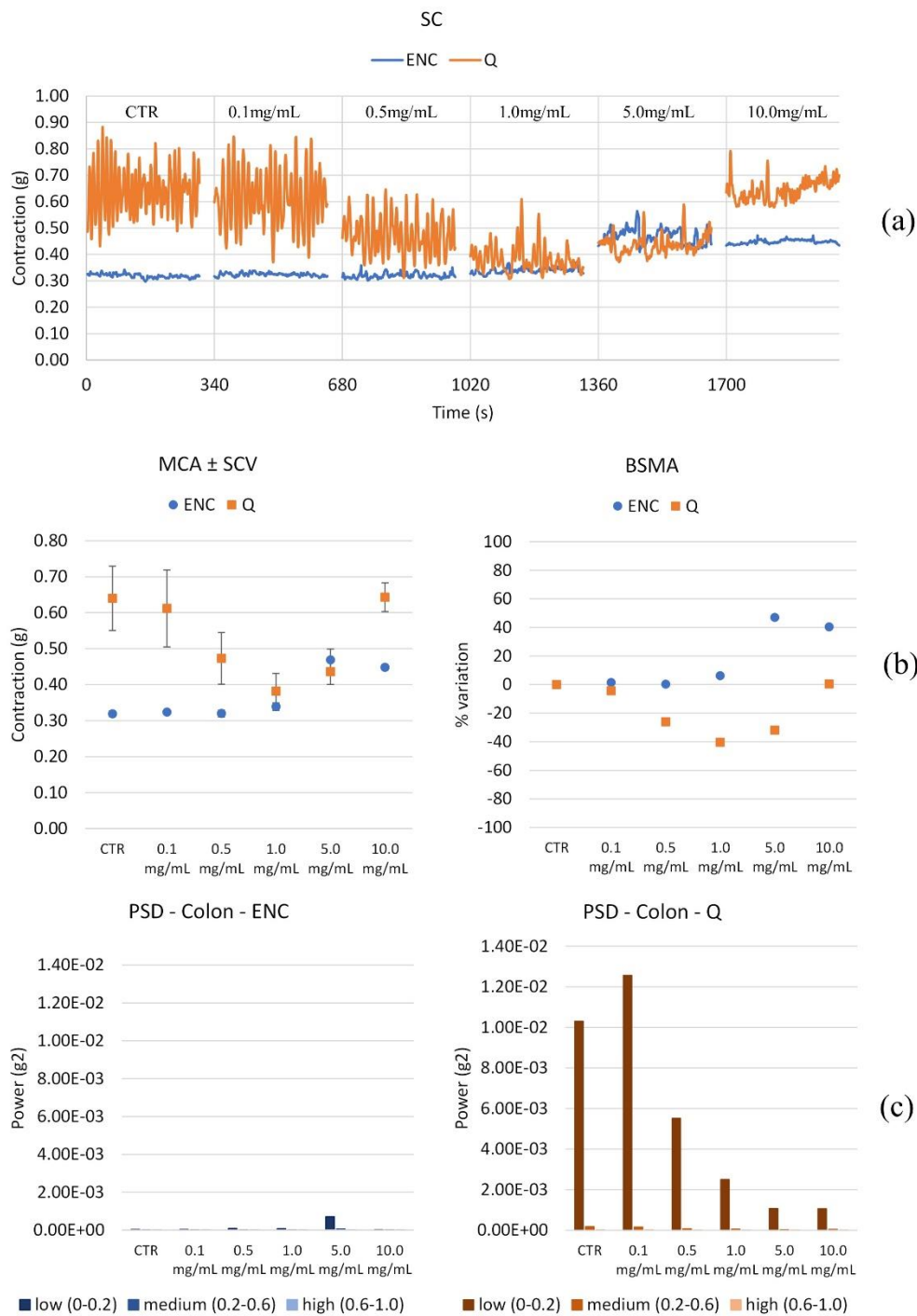


Figure 6. (a) Experimental original recording of the concentration-response curve of ENC (*Castanea sativa* Mill.), and Q (*Schinopsis balansae* Engl.) extract on colon spontaneous contractility (SC). (b) Mean contraction amplitude (MCA) with spontaneous contraction variability (SCV). Basal Spontaneous Motor Activity (BSMA). Zero represents the basal tone and each point is the percent variation from the baseline after cumulative addition of each dose; each value is the mean $\pm$ SEM; when the error bar is not shown, it is covered by the point. (c) Power Spectral Density (PSD).

Figure 7

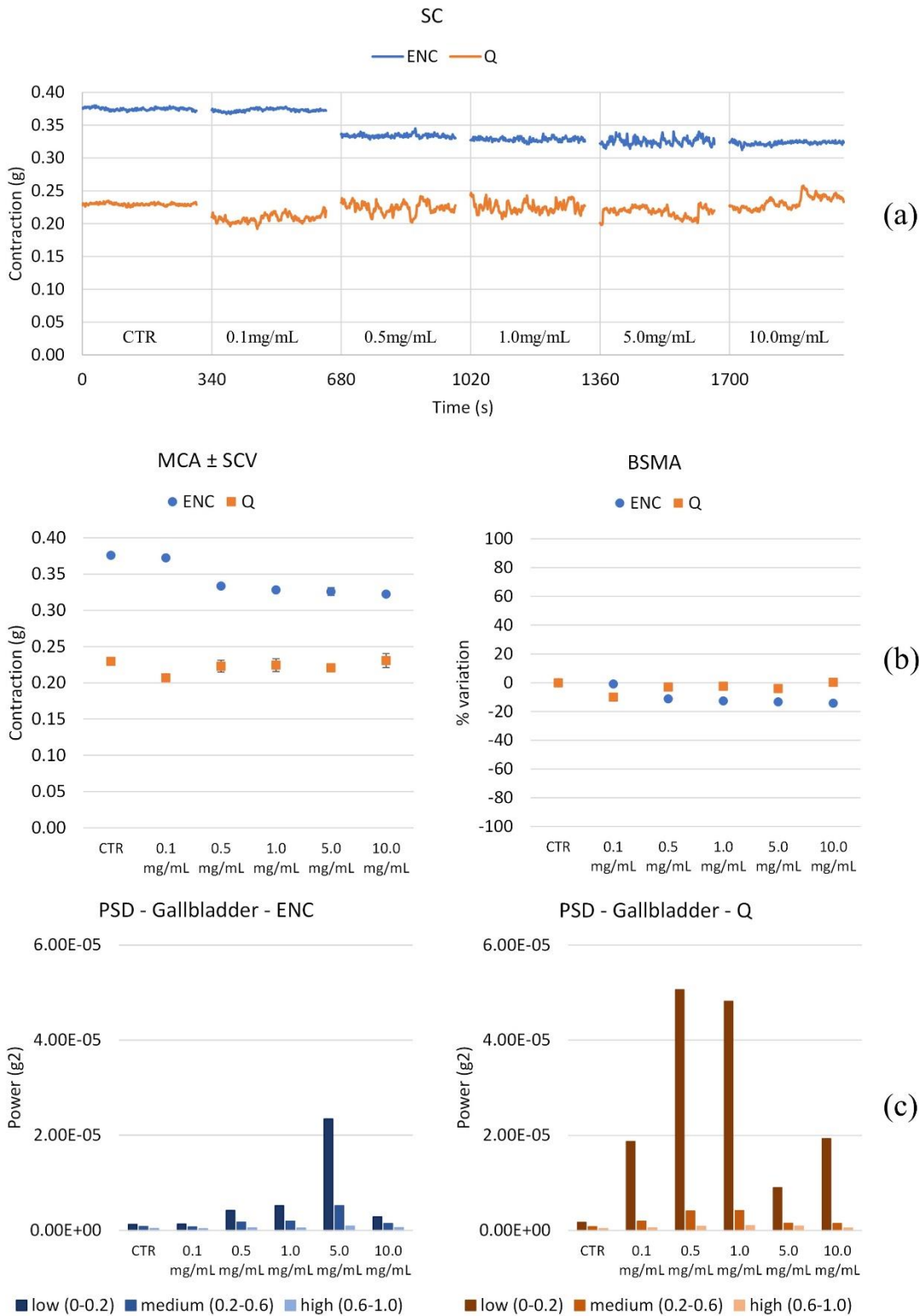


Figure 7. (a) Experimental original recording of the concentration-response curve of ENC (*Castanea sativa* Mill.), and Q (*Schinopsis balansae* Engl.) extract on gallbladder spontaneous contractility (SC). (b) Mean contraction amplitude (MCA) with spontaneous contraction variability (SCV). Basal Spontaneous Motor Activity (BSMA). Zero represents the basal tone and each point is the percent variation from the baseline after cumulative addition of each dose; each value is the mean $\pm$ SEM; when the error bar is not shown, it is covered by the point. (c) Power Spectral Density (PSD).

All the captions were rewritten, as reported	117
Discussion	118
L209 Based on what do the authors say the products are the most promising? compared with what?	119
L 209 became L 248	120
The sentence “are probably the most promising” was changed to “may be promising tools”	121
L215 Which positive effects?	122
L 215 became L 254	123
The positive effects consist of the ability of tannins to affect smooth muscle contractility, to exert antioxidant, antiinflammatory properties and affecting gut microbiota, favouring probiotics growth.	124 125
Materials and methods	126
It is not clear whether the authors made a characterization of the extracts. Some results are reported but no material and method appear in the text.	127 128
Only the “Tannin percentage” obtained by gravimetric analysis is reported. If the authors realized this assay I suggest to include more detailed description of the method.	129 130
Answer: the tannin percentage was obtained by gravimetric analysis of vegetable tanning compounds through the use of Filter-Freiberg Hide Powder Method and it was specified in the text.	131 132
The title of the paragraph where the products are described should be entitled “Materials” or “Chemicals”	133
Answer: Paragraph 4.1 was split in 2 subparagraphs	134
“4.1.1 Chemicals	135
Castanea sativa Mill. Wood (ENC), Schinopsis balansae Engl. hardwood (Q), extracts were used in this study was supplied by SilvaTeam, San Michele di Mondovì, Italy. All of them come from a water extraction process.	136 137
4.1.2 Chemical analyses Tannin percentage was obtained by gravimetric analysis of vegetable tanning agents by using the filter Freiberg-Hide powder method, this is a standard method for the leather industry, but also used in other domains [42].”	138 139 140
L 235 “may favore the”, please proofread grammar.	141
Answer: L 235 became L 292	142
“may favore” was changed to “may favour”	143
Conclusion	144
Conclusions should not contain results. Move table 3 to results.	145
Answer: Table 3 was moved to results, as requested	146

Table 3. “Outlook .on tone modulation by extracts.” Please delete the full stop in the middle of the sentence	147
Answer: This table title was changed to “Outlook of ENC and Q effects in different gastrointestinal tracts.	148
Based on what do you say ENC shows the most promising results? I would include a more extensive and detailed explanation in the discussion section.	149 150
Answer: The whole discussion was revised as requested. In particular we explained that the 2 extracts were compared and they were shown to exert different effects, as shown in table 3, in different gastrointestinal segments. In particular, ENC was shown to have more promising results than Q as it reduces less than Q gastrointestinal contractility, providing effects that may result in a better absorption of nutrients and fats, without inhibiting gut contractions as strongly as Q. Indeed our aim was to evaluate a nutraceutical to be used in non-pathological conditions and not when diarrhoea has already set up. This was reported in the Discussion section, through the following sentence:	151 152 153 154 155 156 157
“Chestnut extract could have a better biological profile, in relation to its application in farm animals, since it influences smooth muscle gastrointestinal contractility in such a way as not to induce constipation, in healthy animals, possibly providing the secondary metabolites endowed with many beneficial effects.” in lines 335 – 338.	158 159 160 161