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# **The link between supplementary tannin level and conjugated linoleic acid (CLA) formation in ruminants: A meta-analysis**

## **A meta-analysis of tannin's level prediction on ruminal biohydrogenation**

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### **Abstract**

This meta-analysis was conducted to predict and assert a way to discover conjugated linoleic acid (CLA) formation in ruminant-derived products as problem solver of human health issues threatened by plant-containing tannins. The objective was to expound, to compare, and to confirm the efficiency of tannins cultivating CLA formation whether using *in vitro* and/or *in vivo* study. A database was created using the ruminants with selectively 26 experiments comprising 683 dietary treatments as explained *in vitro* and *in vivo* methods that were applied as a statistical SAS 9.4 tool. Basically, increasing level of tannins led to an underlying decrease in CLA formation ( $p < 0.001$ ), initially at predicting coefficient determination  $R^2 = 0.193$ ,  $R^2 = 0.929$ , and  $R^2 = 0.549$  for CLA *in vitro*, *in vivo* of CLA milk shift, and *in vivo* of

29 CLA meat precipitation, respectively. *In vitro* may accurately predict to the *in vivo* observation.  
30 Unfortunately, there were no relationship *in vitro* towards *in vivo* observation ( $R^2 < 0.1$ ). It  
31 indicated to be difficult to predict CLA from *in vitro* to *in vivo* separately situations. According  
32 to all studies, the level of tannin's utilization for inhibiting biohydrogenation was not  
33 exceedingly  $>50$  g/kg DM recommended. Secondly, the *in vivo* method was more suitable for  
34 directly observation that concerned in fatty acid transformation.

35

## 36 **Introduction**

37 Nowadays, the consumers have been aware to more selectively in their consumption,  
38 especially ruminant-derived products such a concerning fat composition in milk and meat.  
39 Lourenço, et al [1] reviewed that food for human derived from ruminant product is a high of  
40 Saturated Fatty Acid (SFA) and has lower polyunsaturated fatty acid (PUFA) due to detrimental  
41 condition of human health, including intensified serum low-density lipoprotein (LDL)  
42 cholesterol level, which is a risk factor for coronary heart disease. In previous studies, have  
43 been coined conjugated linoleic acid (CLA) as natural fatty acid (FA) and this FA could solve  
44 aforementioned human problems [1-3]. The predominant isomer of CLA is cis-9, trans-11 18:2,  
45 representing 75–90% of the total CLA in ruminant fat, and trans-7, cis-9 CLA is the second  
46 most prevalent isomer at 3–16% of the total CLA [1, 4] and the trans-11, 18:1 (vaccenic acid)  
47 existence is notable know to support cis-9, trans-11 18:2 [3]. However, producing CLA in milk  
48 and meat is quite difficult because its process invites biohydrogenation respecting to  
49 catalyzation by ruminal microorganisms. For instance, *Butyrifibrio fibrisolvens* was identified  
50 to undertake biohydrogenation of FA and to carry in creating cis-9, trans-11 18:2 and trans-11  
51 18:1 by way of trans-11 18:2 (n-6) [5, 6]. Thus, bacteria acts the fundamental role in FA  
52 biohydrogenation [7] and looking for alternative feed additives from Phytochemicals [8] as anti-  
53 microbial could be greater option to increase CLA in ruminant products.

54 Essential oils are commonly supplementation derived from plant and marine product.  
55 Their function exactly had many modes bringing ruminal bacteria N down [9] and inhibited  
56 survival of *Butivibrio fibrisolvens* and *Butivibrio proteoclasticus* community on  
57 biohydrogenation[10]. The effective of essential oils had variable impacts on ruminal  
58 fermentation [11], it might be believed in depend on source extraction, method, dose, basic diet,  
59 pH and preliminary period of microorganism to adapt essential oils. Subsequently, forage  
60 feeding is used gaining long chain of PUFA as galacto-, sulfo-, and phospholipid that could  
61 exert keeping PUFA long time on biohydrogenation. Besides, ionosphere feed additive namely  
62 saponins and tannins is coming to deserve attention as antimicrobial properties. Li, et al [12],  
63 ability of tannin supplementation had a broadly distortion of rumen microbiota, thereby being  
64 useful to shift rumen performance. Another, saponins concerned to inhibit methane emission  
65 and lower biohydrogenation because of defaunation function leading to protozoa-lipid  
66 population decrease [9]. On other hand, tannins had a greater impressive mode, particularly  
67 antimicrobial behavior to assert *Clostridium proteoclasticus* converting trans-11, 18:1 to 18:0  
68 form [10]. Consequently, tannins could be appreciated as a temporary fraction to improve CLA  
69 production in FA composed manipulation of ruminal fermentation.

70 Tannins including condensed and hydrolyzed forms have expressed widely antimicrobial  
71 properties in rumen studies. In previous years, Jayanegara, et al [13], conducted meta-analysis  
72 with collecting data from *in vitro* and *in vivo* studies that supplementing feed-containing tannins  
73 in rumen feedstuff diminished methane level and affected to palatability of ruminant. Also,  
74 Jerónimo, et al [14], reviewed chemical structure of tannins behaved rough effect on animal  
75 performance and the quality of their products (meat and milk) particularly on the fatty acid  
76 profile, oxidative stability, and organoleptic properties. In these two publications explained  
77 valuable tannins, edible usages, and potential functions separately. Although, no one even in  
78 single chapter addressed a relationship of tannin supplementation in rumen diet towards to

79 biohydrogenation approaching with the meta-analysis technique. The clear-cut method whether  
80 using *in vitro* and/or *in vivo* to provide a prerequisite is also needed. Hopefully, the result of  
81 present study could be useful for animal science, animal nutrition, and biotechnology expertise.  
82 Therefore, the objectives were (i) to expound the effectiveness of tannins modulating CLA  
83 formation, (ii) to study comparison of gained result based on *in vitro* and *in vivo* methods, (iii)  
84 to confirm the relationship between *in vitro* and *in vivo* studies applying the meta-analysis as a  
85 statistical tool.

86

## 87 **Methods**

### 88 **Search strategy and selection criteria**

89 A database created from experiments which the dietary tannin concentrations and CLA  
90 properties were concerned touching closer to PRISMA (Preferred Reporting Items for  
91 Systematic Reviews and Meta-Analyses) [15], see Fig 1. These data were gathered on the ISI  
92 Web of Science (recently form in ISI Web of Knowledge) database using “conjugated linoleic  
93 acid,” “biohydrogenation”, “rumen,” “tannin,” “meat,” “milk,” “*in vivo*,” and “*in vitro*” as  
94 keywords from October 31, 2016 to March 23, 2019. Title/abstract, topic and keyword search  
95 terms were used in combination. Results were limited to trials published in English (S1 Table).  
96 For further consideration, the results were touched with single search in relevant studies and  
97 reviews. Endnote (Thompson ISI Research-Soft, Philadelphia, PA, US) was used to repository  
98 the relevant articles and remove duplicate articles.

99

### 100 **Study criteria, quality assessment, and data extraction**

101 Studies were included if they met the following criteria: (1) the study design was an *in*  
102 *vitro*; (2) the study design was an *in vivo*; (3) Object used ruminants: cow, goat, sheep in dairy  
103 or meat product (4) relevant data was retrievable; and (5) the studies were published after 1  
104 December 2008. Authors were contacted by e-mail and ResearchGate provider, if data had

105 questionable. If that was unsuccessful, references were excluded on account of inaccessibility  
106 of data.

107 The raw data were strictly screened and accepted in similar calculating unit per parameter,  
108 e.g., g/kg FAME (fatty acid methyl ester) and g/kg DM (dry matter) for all FA and tannin level,  
109 respectively. Finally, the comprehensive database consisted of 683 dietary treatments in 26  
110 experiments as explanation in Table 1 (*in vitro* experiments) and Table 2 (*in vivo* experiments).  
111 In addition, the sources were collected even deriving from individually publication. The  
112 database was picked selectively into two categories based on different methods or systems  
113 applied in the experiments. As a result, there were *in vitro* batch culture (10 experiments/356  
114 treatments) and *in vivo* experiments (16/327) with 10 experiments concerning CLA level from  
115 milk source and 6 others from intra muscular fatty acid in meat source, completing with 2  
116 experiments conducted both *in vitro* and *in vivo* on the same time. The Cochrane Reviewer's  
117 Handbook 4.2 was used to assess the risk of bias.

118

## 119 **Statistical analysis**

120 The analysis of the data assembled in the database was conducted by a statistical meta-  
121 analysis approach [13, 16, 17]. Using the MIXED procedure of SAS 9.4 version [18], the  
122 following model was applied:

$$123 \quad Y_{ij} = P_0 + P_1Q_{ij} + R_i + \pi_iQ_{ij} + e_{ij} \quad (1)$$

124 where  $Y_{ij}$  = dependent variable,  $P_0$  = overall intercept across all experiments (fixed effect),  $P_1$ =  
125 linear regression coefficient of Y on X (fixed effect),  $Q_{ij}$  = value of the continuous predictor  
126 variable (supplementary tannin level),  $R_i$  = random effect of experiment  $i$ ,  $\pi_i$  = random effect  
127 of experiment  $i$  on the regression coefficient of Y on X in experiment  $i$  and  $e_{ij}$  = the deniable  
128 residual error. To input the CLASS statement, the variable 'REFERENCENO' was subjected  
129 without any quantitative information. Additionally, data were calculated by the number of  
130 animal replications in each experiment [18] and scaled to 1 to avoid misconception regarding

131 unequal variance among experiments. In a fixed-effects model, a small study was considerably  
132 ignored, though, considerable weight was adjusted to a large study (based on number of  
133 measurements).

134 Outliers were identified by examining mixed procedure with maximum-likelihood (ML).  
135 For illustrating, it used METHOD=ML; COVTEST; PARMs statement followed by the  
136 EQCONS=2 option. An unstructured variance–covariance matrix (type = un) was confirmed as  
137 the random part of the model. Also, the comparison between CLA number from milk and meat  
138 source could not compare directly. It would be possible comparing total data including covering  
139 from *in vitro* and *in vivo* observations. Incompleteness of selected data on involving variables,  
140 meta analyses were technically performed based on the data available for individual variables.

141

142 **Fig 1. Modified flow chart of the selection process for the eligible studies.**

143

## 144 **Results**

### 145 **Search results and bias assessment**

146 As depicted in Fig 1, identified articles had 11038 potentially relevant studies. Articles  
147 were checked compressing at 26 studies, see table 1 and 2. Twenty-six studies had performed  
148 the critically information, while 2 experiments conducted both *in vitro* and *in vivo* on the same  
149 time (Szczechowiak, et al [5] and Toral, et al [19]). According to Cochrane Reviewer's  
150 Handbook 4.2 to assess risk of bias (S1 Fig). The high-risk studies were disclosed. Five of them  
151 had a low risk of bias.

152

### 153 **The effectiveness level of tannins**

154 The meta regression strictly between dietary tannins and CLA levels from the *in vitro*  
155 batch culture experiment and the *in vivo* experiment is presented in Table 3 and Table 4,  
156 respectively. The optimum level of tannins for modulating CLA level coming along a nurture

157 rumen fermentation was predicted around 0.1-50 g/kg DM. Regardless of tannin type, the tough  
158 natural chemists from tannins provoked the CLA going down gradually ( $p<0.001$ ) of both  
159 studies in *in vivo* CLA milk shift (Fig 2) with an  $R^2$  of 0.929 and *in vivo* CLA meat precipitation  
160 (Fig 3) with an  $R^2$  of 0.549. However, supplementing a surge of tannin level increased the CLA  
161 level in *in vitro* study, yet, the efficiency of tannin acted dubious. Truly, a rising of CLA trend  
162 ( $p<0.001$ ) was followed by a linear relationship rather than a quadratic response (Fig 4) with  
163 an  $R^2$  of 0.193.

164

## 165 **The regression of method application**

166 The regression relationships between the *in vitro-in vivo* of CLA milk form is depicted in  
167 Fig 5 and *in vitro-in vivo* of CLA meat deposition shown in Fig 6. These relationships were  
168 expressed by a linear relationship rather than a quadratic response. Clearly, there were no  
169 relationship among them ( $R^2<0.1$ ).

170

171 **Fig 2. The linear relationship between dietary tannins (g/kg DM) and CLA milk shift**  
172 **(g/kg FAME) using *in vivo*.**

173

174 **Fig 3. The linear relationship between dietary tannins (g/kg DM) and CLA meat shift**  
175 **(g/kg FAME) using *in vivo*.**

176

177 **Fig 4. The linear relationship between dietary tannins (g/kg DM) and CLA (g/kg FAME)**  
178 **using *in vitro*.**

179

180 **Fig 5. The relationship between *in vitro* and *in vivo* CLA milk form.**

181

182 **Fig 6. The relationship between *in vitro* and *in vivo* CLA meat form.**

**Table 1. Data tabulation of *in vitro* experiments**

No exp.	Reference	<i>In vitro</i> method <sup>a</sup>	Donor inocula	Basal feed <sup>b</sup>	Tannins source <sup>c</sup>	Tannin level (g/kg DM) <sup>d</sup>	Gas sampling (h)	Fatty acid method <sup>e</sup>
1	Vasta, et al [20]	GBI	Cow (Friesian–Holstein)	Hay and Hay plus	<i>Ceratonia siliqua</i> (CT), <i>Acacia cyanophylla</i> (CT), <i>Schinopsis lorentzii</i> (CT)	0.06-0.01	12	FAME
2	Toral, et al [19]	HGT	Sheep (Ewe)	TMR, Alfafa hay: Concentrate (40:60) + 20g sunflower/ kg DMI	Quebracho (CT) + Chesnut (HT)	10	24	FAME
3	Jayanegara, et al [21]	HGT	Cow (Brown Swiss)	Hay (white clover), ryegrass and concentrate	<i>Acacia mangium</i> , <i>Acacia villosa</i> , <i>Albizia falcataria</i> , <i>Artocarpus heterophyllu</i> , <i>Calliandra calothyrsus</i> , <i>Canna indica</i> , <i>Carica papaya</i> , <i>Clidemia hirta</i> , <i>Cycas rumphii</i> , <i>Erythrina orientalis</i> , <i>Eugenia aquea</i> , <i>Hibiscus tiliaceus</i> , <i>Ipomoea batatas</i> , <i>Lantana camara</i> , <i>Leucaena diversifolia</i> , <i>Leucaena leucocephala</i> , <i>Manihot esculenta</i> , <i>Melia azedarach</i> , <i>Mimosa invisa</i> , <i>Morinda citrifolia</i> , <i>Myristica fragrans</i> , <i>Paspalum dilatatum</i> , <i>Persea Americana</i> , <i>Pithecellobium jiringa</i> , <i>Psidium guajava</i> , <i>Sesbania grandiflora</i> , <i>Swietenia mahagoni</i> .	2-220	24	FAME



No exp.	Reference	<i>In vitro</i> method <sup>a</sup>	Donor inocula	Basal feed <sup>b</sup>	Tannins source <sup>c</sup>	Tannin level (g/kg DM) <sup>d</sup>	Gas sampling (h)	Fatty acid method <sup>e</sup>
4	Rana, et al [2]	HGT	Goat (Alpine × Beetal)	Forage and concentrate	<i>Terminalia chebula</i> (CT)	1.06 and 3.18	24	FAME
5	Jayanegara, et al [22]	HGT	Cow (Brown Swiss)	Clover-ryegrass hay and concentrate	<i>Poa alpina</i> (HT), <i>Achillea millefolium</i> (HT), <i>Alchemilla xanthochlora</i> (HT), <i>Capsella bursapastoris</i> (HT), <i>Carum carvi</i> (HT), <i>Chrysanthemum adustum</i> (HT), <i>Crepis aurea</i> (HT), <i>Plantago atrata</i> (HT), <i>Rhinanthus alectorolophus</i> (HT), <i>Rumex arifolius</i> (HT), <i>Anthyllis vulneraria</i> (HT), <i>Hedysarum hedysaroides</i> (HT), <i>Trifolium badium</i> (HT), <i>Castanea sativa</i> (HT), <i>Fraxinus excelsior</i> (HT), <i>Sambucus nigra</i> (flowers) (HT).	1-78	24	FAME
6	Minieri, et al [23]	HGT	Sheep (Ewe)	Forage and concentrate	Quebracho (CT)	49	24	FAME
7	Carreño, et al [24]	BCI	Sheep (Ewe)	TMR, Forage: Concentrate (50:50)	Chesnut (HT), Oak (HT), Quebracho (CT).	20-80	24	FAME

No exp.	Reference	<i>In vitro</i> method <sup>a</sup>	Donor inocula	Basal feed <sup>b</sup>	Tannins source <sup>c</sup>	Tannin level (g/kg DM) <sup>d</sup>	Gas sampling (h)	Fatty acid method <sup>e</sup>
8	Ishlak, et al [25]	BCI	Cow (Holstein)	Forage: Concentrate (55:45)	Quebracho (CT)	100	24	GC as described by Jenkins, et al [26]
9	Toral, et al [27]	BCI	Sheep (Ewe)	Hay (Alfafa)	<i>Onobrychis viciifolia</i> (CT)	49	24	FAME
10	Szczechowiak, et al [5]	Bag incubate of RUSITE C	Cow (Polish Frisien Holstein)	PMR, silage and concentrate	<i>Vaccinium vitisidaea</i> (CT)	4.5	24	FAME

184 <sup>a</sup>GBI = glass bottle incubation; BCI = batch culture incubation; HGT = Hohenheim gas test.

185 <sup>b</sup>PMR = Partial Mixed Ration; TMR = total mixed ration; DMI = dry matter intake.

186 <sup>c</sup>CT = condensed tannins; HT = hydrolysable tannins.

187 <sup>d</sup>DM=dry matter.

188 <sup>e</sup>GC = gas chromatograph; FAME = fatty acid methyl ester.

189

**Table 2. Data tabulation of *in vivo* experiments.**

No Exp.	Reference	Species	Basal feed <sup>a</sup>	Tannins source <sup>b</sup>	Tannin level (g/kg DM) <sup>c</sup>	Fatty Acid Method <sup>d</sup>	Adaptation period/Long treatment (day) <sup>e</sup>	Milking (time/day)/Slaughtered period (day of age)
11	Vasta, et al [28]	Goat (Comisana Lamb)	Alfafa and concentrate with grazing	Carob	42	FAME	9/55	105
12	Vasta, et al [29]	Goat (Comisana Lamb)	Vetch and concentrate with grazing	Quebracho (CT) + Chesnut (HT),	40.4 and 40.6	FAME	7/60	105
13	Vasta, et al [30]	Goat (Comisana Lamb)	Vetch and concentrate with grazing	Quebracho (Schinopsis lorentzii) (CT)	1	FAME	NS/60	105
14	Cabiddu, et al [4]	Sheep (Sarda ewe)	Grazing, Pasture sulla	<i>Flowering sulla (Hedysarum coronarium L.)</i> (CT)	200	FAME	NS/NS	2
15	Vasta, et al [31]	Goat (Comisana Lamb)	Alfafa hay and concentrate with grazing	Quebracho (CT)	95.7	FAME	7/70	122
16	Toral, et al [19]	Sheep (Assaf ewe)	Alfafa hay: Concentrate (40:60) + 20g sunflower/ kg DMI	Quebracho (CT) + Chesnut (HT)	10	FAME	14/30	2
17	Dschaak , et al [32]	Cow (Holstein)	Forage and concentrate (59:41)	Quebracho (CT)	0.801-1.801	FAME	14/7	2
18	Staerfl, et al [33]	Bull (Brown Swiss×Limousin crossbred)	PMR, Maize silage and concentrate	<i>Acacia mearnsii tannins</i> (CT)	700	FAME	23/280	304

No Exp.	Reference	Species	Basal feed <sup>a</sup>	Tannins source <sup>b</sup>	Tannin level (g/kg DM) <sup>c</sup>	Fatty Acid Method <sup>d</sup>	Adaptation period/Long treatment (day) <sup>e</sup>	Milking (time/day)/Slaughtered period (day of age)
19	Marume, et al [34]	Goat (Xhosa lop-eared)	Hay and concentrate with grazing	<i>Acacia karoo</i>	200	FAME	30/60	210
20	Kälber, et al [35]	Cow (Frisien and Brown Swiss)	TMR, Rygrass and concentrate	Buchwheat vegetative, Buchwheat, Chicory vegetative, Chicory e, Phacelia vegetative, Phacelia,	0.6-0.48	FAME	24/26	2
21	Willems, et al [36]	Lamb (Engadine and Valaisian Black Nose sheep)	Ryegrass-clover pasture (grazing)	Swards	0.3-1.64	FAME	30/63	183
22	Buccioni , et al [37]	Sheep (Comisana ewe)	PMR, Hay and concentrate	Chesnut (HT)	52.8	FAME	15/30	2
24	Miri, et al [6]	Dairy goats (Alpine × Beetal)	Forage and concentrate	Cumin	1.27-25.3	FAME	21/30	2
25	Girard, et al [38]	Cow (Holstein)	TMR, a mixture of grass hay (86:10:4 of grass, legumes, and other species, respectively)	Sainfoin (CT), BirdSfooT trefoil bull (CT), BirdSfooT trefoil polom. (CT),	120-691	FAME	24/25	2

No Exp.	Reference	Species	Basal feed <sup>a</sup>	Tannins source <sup>b</sup>	Tannin level (g/kg DM) <sup>c</sup>	Fatty Acid Method <sup>d</sup>	Adaptation period/Long treatment (day) <sup>e</sup>	Milking (time/day)/Slaughtered period (day of age)
26	Szczechowiak, et al [5]	Cow (Polish Frisien Holstein)	Mix silage and concentrate	<i>Vaccinium vitis idaea</i> (CT)	32.2-48.3	FAME	21/5	2

192 <sup>a</sup>PMR = Partial Mixed Ration; TMR = total mixed ration; DMI = dry matter intake.

193 <sup>b</sup>CT = condensed tannins; HT = hydrolysable tannins.

194 <sup>c</sup>DM=dry matter.

195 <sup>d</sup>FAME = fatty acid methyl ester.

196 <sup>e</sup>NS= not spesific.

197

**Table 3. The predicting equation of *in vitro* batch culture experiments<sup>a</sup>**

Response parameter <sup>b</sup>	N <sup>c</sup>	Parameter estimation <sup>d</sup>							
		Intercept	SE intercept	p intercept	Slope	SE slope	P slope	RMSE	R <sup>2</sup>
FA supplementation (g/kg of total FA)									
C18:3 n-3	351	0.0263	0.0152	0.0852	0.00003	0.0002	0.8632	0.0072	0.5568
C18:2 n-6	342	0.0593	0.0245	0.0160	1.97E-6	0.0004	0.9961	0.0130	0.5770
C18:1 n-9	264	0.0432	0.0232	0.0633	0.00001	0.0009	0.9842	0.0036	0.7907
Gas production (mL/g OM)	70	0.0341	0.1831	0.8528	0.0009	0.0026	0.7205	0.0065	0.4476
Total VFA (mmol/L)	81	-0.1092	0.2902	0.7077	0.0126	0.0056	0.0243	0.1111	0.5787
C <sub>2</sub>	81	0.3339	0.0679	<0.0001	0.0027	0.0031	0.4463	0.9799	0.4527
C <sub>3</sub>	81	0.5183	0.0353	<0.0001	-0.0236	0.0070	0.0007	0.5213	0.8407
C <sub>4</sub>	81	0.4562	0.0185	<0.0001	-0.0058	0.0063	0.3613	0.6118	0.9290
C <sub>5</sub>	76	0.1249	0.0297	<0.0001	0.1034	0.0293	0.0004	2.4934	0.6688
Iso-C <sub>4</sub> +Iso-C <sub>5</sub>	65	0.0583	0.0268	0.0334	0.0087	0.0031	0.0045	0.1261	0.6534
FA profile (g/kg FAME)									
Cis-9, trans-11, 18:2 (CLA)	353	0.1347	0.0233	<0.0001	-0.0009	0.0071	0.9896	2.3871	0.1929
Trans-11 18:1	353	0.0430	0.0301	0.1535	0.0009	0.0016	0.5451	0.0065	0.5649
C18:0	353	0.0563	0.0273	0.0397	-0.0001	0.0006	0.8010	0.0057	0.7830
SFA	310	0.0317	0.0431	0.4623	0.0003	0.0006	0.5622	0.0232	0.1353
MUFA	306	0.0650	0.0410	0.1136	0.0009	0.0014	0.5244	0.0340	0.3011
PUFA	301	0.1702	0.0412	<0.0001	-0.0002	0.0003	0.5034	0.1169	0.3646

200 <sup>a</sup>Outcomes are averages deriving from tabulated data in table 1 calculating using proc mixed.

201 <sup>b</sup>C<sub>2</sub> = acetate; C<sub>3</sub> = propionate; C<sub>4</sub> = butyrate; C<sub>5</sub> = valerate; VFA = volatile fatty acids; FA = fatty acids; FAME = fatty acid methyl esters; CLA = conjugated linoleic acid;  
 202 SFA = saturated fatty acids; MUFA = mono-unsaturated fatty acids; PUFA = poly-unsaturated Fatty Acids; DM = dry matter; OM= organic matter.

203 <sup>c</sup>N= total data used.

204 <sup>d</sup>SE = Standard error; RMSE= residual mean square error; R<sup>2</sup>= coefficient of determination.

205

**Table 4. The predicting equation of *in vivo* batch culture experiments<sup>a</sup>**

Response parameter <sup>b</sup>	N <sup>c</sup>	Parameter estimation <sup>d</sup>							
		Intercept	SE intercept	p intercept	Intercept	SE slope	P slope	Intercept	R <sup>2</sup>
FA supplementation (g/kg of total FA)									
C18:3 n-3	276	0.3507	0.0328	<0.0001	-0.0040	0.0008	<0.0001	0.8983	0.4374
C18:2 n-6	278	0.2603	0.0206	<0.0001	-0.0071	0.0013	<0.0001	0.5895	0.4519
C18:1 n-9	247	0.2874	0.0438	<0.0001	-0.0093	0.0024	<0.0001	0.7079	0.2651
Total VFA (mmol/L)	73	0.3462	0.4800	0.4731	-0.0022	0.0050	0.6663	0.0192	0.4513
C <sub>2</sub>	73	0.0753	0.0428	0.0826	0.0007	0.0008	0.4166	0.0408	0.7222
C <sub>3</sub>	73	0.4125	0.0538	<0.0001	-0.0174	0.0051	0.0007	0.1785	0.7889
C <sub>4</sub>	73	0.4404	0.0621	<0.0001	-0.0338	0.0084	<0.0001	1.4362	0.5928
C <sub>5</sub>	47	0.4218	0.0211	<0.0001	0.0714	0.0316	0.0238	0.7399	0.9571
Iso-C <sub>4</sub> + Iso-C <sub>5</sub>	40	0.6673	0.0431	<0.0001	-0.1248	0.0212	<0.0001	1.2507	0.8956
Desaturation index									
C18:2 cis-9 trans-11:C18:1 trans-11	60	-0.2272	0.0191	<0.0001	24.6657	1.5204	<0.0001	4.7579	0.5538
FA profile in milk (g/kg FAME)									
Cis-9, trans-11 18:2 (CLA)	281	0.1704	0.0129	<0.0001	0.0967	0.0070	<0.0001	1.6716	0.9289
Trans-11 18:1	283	0.0676	0.0155	<0.0001	0.0115	0.0027	<0.0001	0.2682	0.3880
C18:0	283	0.2181	0.0503	<0.0001	0.0015	0.0016	0.3603	0.2653	0.3407
SFA	306	0.4411	0.0755	<0.0001	-0.0046	0.0013	0.0004	0.4115	0.3465
MUFA	295	-0.0257	0.0577	0.6560	0.0083	0.0012	<0.0001	0.4716	0.4552
PUFA	284	0.2370	0.0326	<0.0001	0.0014	0.0008	0.0740	0.7109	0.4594
FA profile in longissimus dorsi muscle (g/kg FAME)									
Cis-9, trans-11, 18:2 (CLA)	172	0.0193	0.0651	0.7682	0.0009	0.0330	0.9771	0.0026	0.5491
Trans-11, 18:1	172	0.0102	0.0442	0.8183	-0.0003	0.0068	0.9706	0.0005	0.4547
C18:0	172	-0.0035	0.0994	0.9720	0.0023	0.0053	0.6684	0.0026	0.3705
SFA	172	0.1035	0.1265	0.4146	-0.0011	0.0031	0.7135	0.0088	0.3773
MUFA	172	0.0458	0.1912	0.8109	0.0004	0.0046	0.9270	0.0006	0.8220
PUFA	172	0.0517	0.1166	0.6583	0.0005	0.0032	0.8701	0.0006	0.8347

<sup>a</sup>Outcomes are averages deriving from tabulated data in table 1 calculating using proc mixed.

209 <sup>b</sup>C<sub>2</sub> = acetate; C<sub>3</sub> = propionate; C<sub>4</sub> = butyrate; C<sub>5</sub> = valerate; VFA = volatile fatty acids; FA = fatty acids; FAME = fatty acid methyl esters; CLA = conjugated linoleic acid;  
210 SFA = saturated fatty acids; MUFA = mono-unsaturated fatty acids; PUFA = poly-unsaturated Fatty Acids; DM = dry matter; OM= organic matter.  
211 <sup>c</sup>N= total data used.  
212 <sup>d</sup>SE = Standard error; RMSE= residual mean square error; R<sup>2</sup>= coefficient of determination.



## 213 Discussion

214 In this meta-analysis provided underlying prediction and perspective in other tannin ways  
215 such a manipulating biohydrogenation to compulsive CLA production massively. As shown in  
216 Table 3, dietary tannin affected as statistically regression of the CLA components in milk form  
217 ( $R^2 > 0.9$ ), however, not in meat precipitation ( $R^2 < 0.9$ ) without collecting bias data. These results  
218 were indirectly as same as earlier meta-analysis predicting dietary tannin level on rumen diet<sup>12</sup>.  
219 Enhancing effort to modulate biohydrogenation is rough and tricky, wherein choose a suitable  
220 method to approve. More elaborating reasons, addressed by Lourenço, et al [1], a role for  
221 manipulating biohydrogenation was difficult because of inviting systematically isomerization  
222 through decreasing hydrogen supply and assessing stearic (C18:0) bacteria. Unfortunately, this  
223 meta-analysis was at dull in rumen bacterial biohydrogenation in order to limitation of public-  
224 access records.

225 One way to obtain information of this study was merely understanding to input a  
226 sufficiency feedstuff, particularly fiber and fatty acid source, to start fermented nutrient in  
227 creating gas production including hydrogen accumulation that could be as references  
228 continuously on biohydrogenation. Castro, et al [39], shown diet containing more fat source  
229 increased desaturation index, though there was lower regression number of this regard ( $R^2 < 0.9$ ),  
230 see table 4. In addition, whether in *in vitro* and/or *in vivo* demonstrated low regression ( $R^2 < 0.9$ )  
231 of fatty acid role on predicting tannin properties, see table 3 and 4. Another, trans 11 C18:1  
232 (vaccenic acid) were not affected ( $p < 0.001$ ,  $R^2 < 0.9$ ) by dietary tannin in all methods. Hence,  
233 there was a relationship between FA supplementation with desaturase index on ruminal  
234 biohydrogenation.

235 Furthermore, Jayanegara, et al [13], the regression of tannin effect on rumen metabolism  
236 and its methane loss provoked a lesser biohydrogenation indirectly, through diminishing a  
237 hydrogen (H<sub>2</sub>) supply and contribution of volatile fatty acid (VFA's). This was corresponding

238 to a higher propionate catching H<sub>2</sub> down leading to greater biohydrogenation failure. In this  
239 meta-analysis, the propionate was interrupted (p<0.001) by tannin supplementation with a  
240 tantamount R<sup>2</sup> value. In same way, Dschaak , et al [32], reported supplementary condensed  
241 tannin in different forage levels interpreted an increase of propionate, yet, no affection for  
242 distributed CLA. However, Buccioni , et al [37], declining propionate at 34.3% against control  
243 diet tending to increase of CLA formation around 24.2% in milk production, when dairy ewes  
244 fed quebracho as tannin-containing feedstuff. It might be tannin form inducing the different  
245 sub-active compound inside leading to the different affections and this mechanism would be  
246 only running on fat metabolism persuading a recycling lively organ such a liver. Although, *in*  
247 *vitro* study reported in different way in this study, see table 3.

248 Comparison of selectively differential methods by present of CLA fractions are possibly  
249 corresponded each other with similar units of measurement. To be recognized, the *in vitro* bath  
250 culture method ran dissimilar towards to the *in vivo* method in this study. Nevertheless, the  
251 media flow out substantially carried on their metabolism and there was lively absorption of the  
252 rumen properties directly on the process. Remember, in this meta-analysis concern, one unit  
253 was presenting on the graph by CLA (g/kg FAME) to clarify the relationship between CLA *in*  
254 *vitro* relating to CLA milk *in vivo* (Fig 5) and CLA *in vitro* relating to CLA meat *in vivo* (Fig  
255 6). Astoundingly, both of their relationships had expressed as to be poor regression (R<sup>2</sup> <0.5)  
256 [40]. Thus, it could be clarified that being challenging to predict from *in vitro* observation to *in*  
257 *vivo* situations accurately on CLA property determination of the ruminants<sup>5,40</sup>, and/or field  
258 objective close to FA measurement<sup>41</sup>. It was known in advance that the *in vitro* observation  
259 presenting with a current limitation, especially to extrapolate how systematically synthesizing  
260 biohydrogenation of fatty acid was.

261 Utterly, dietary tannins supplementation to ruminants sentenced multiparous benefits,  
262 especially at CLA production. The most significant findings in this study were that the

263 ruminants achieving tannin-containing diet altered rumen fermentation leading to direct and  
264 indirect effects on biohydrogenation. Yet, the suitable method was considered as perquisite trial  
265 whether *in vitro* and *in vivo* studies. Therefore, dietary tannin may change other specifically  
266 parameters, for instance behavior of gene expressions for further investigations needed.

267

## 268 **Conclusion**

269 Coming with sizeable data from the valid publications, this meta-analysis provided a  
270 prediction of suitable plant-containing tannin level in ruminal diet and their application facing  
271 a fit method design for developing CLA formation on biohydrogenation. The optimum level of  
272 tannins was predicted around 0.1-5.0 g/kg DM. Basically, adjusting level of tannins declined  
273 the CLA number. Secondly, the *in vivo* method was more suitable for directly observation that  
274 concerned in FA transformation. Unless, using the *in vitro* observation was easier, cheaper, and  
275 more edible presenting with a current limitation, particularly to understand the full outcome of  
276 systematically synthesizing FA on biohydrogenation.

277

## 278 **Supporting information**

279 **S1 Table. Full electronic search strategy for ISI Web of Knowledge.**

280

281 **S1 Fig. Bias assessment using Cochrane Reviewer's Handbook 4.2.**

282

283 **S1 Checklist. PRISMA checklist.**

284

## 285 **Acknowledgment**

286 Authors would like to say thanks to Laurence V. Madden, Amonrat Molee, and Jan  
287 Thomas Schonewille for preparing database design.

## 288 **Author contribution**

289 **Conceptualization, methodology, investigation, resources, data curation and writing—**

290 **original draft preparation:** Rayudika Aprilia Patindra Purba.

291 **Funding acquisition, project administration and writing—review and editing:** Rayudika

292 Aprilia Patindra Purba, Pramote Paengkoum, Siwaporn Paengkoum.

293

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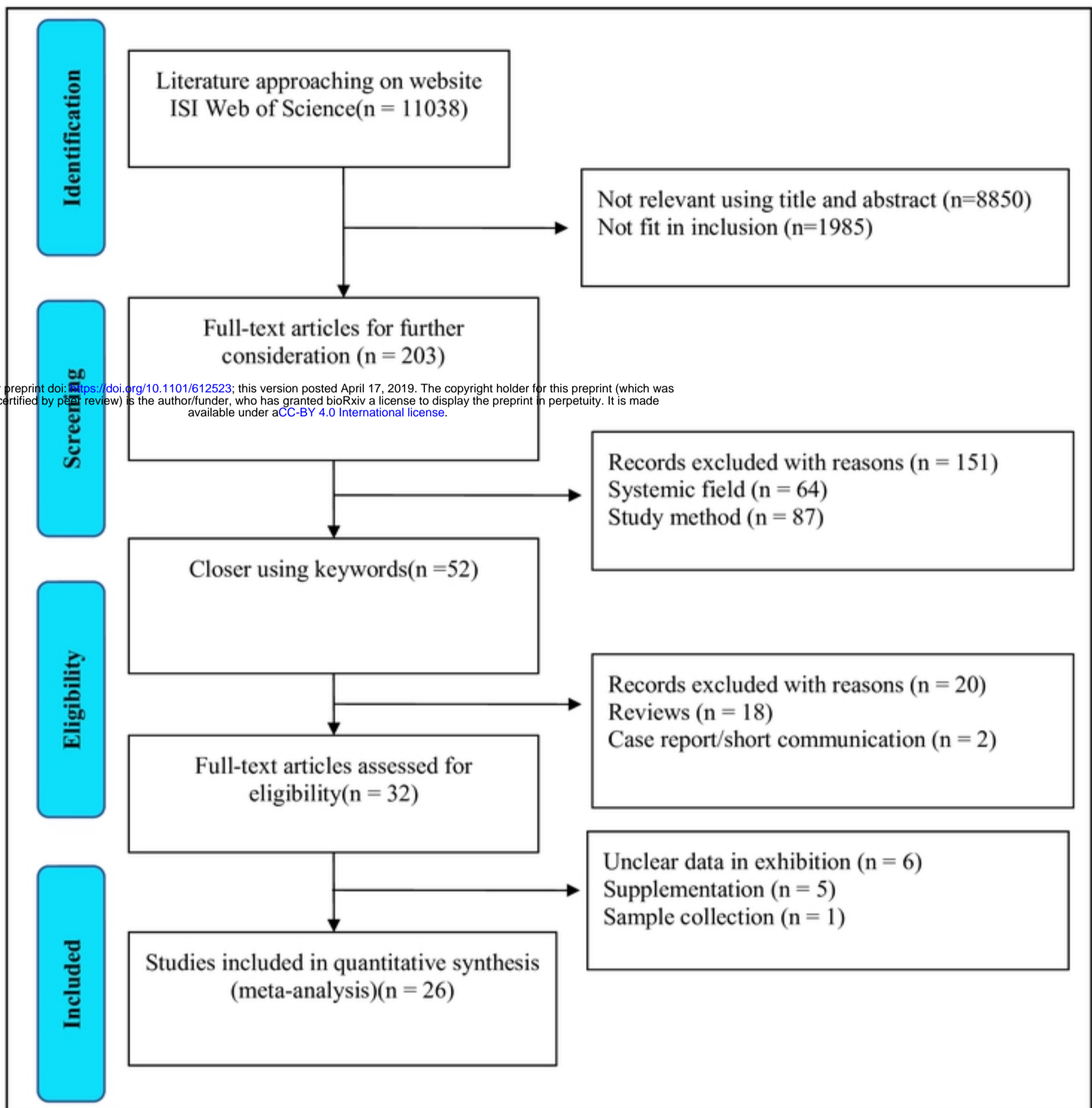
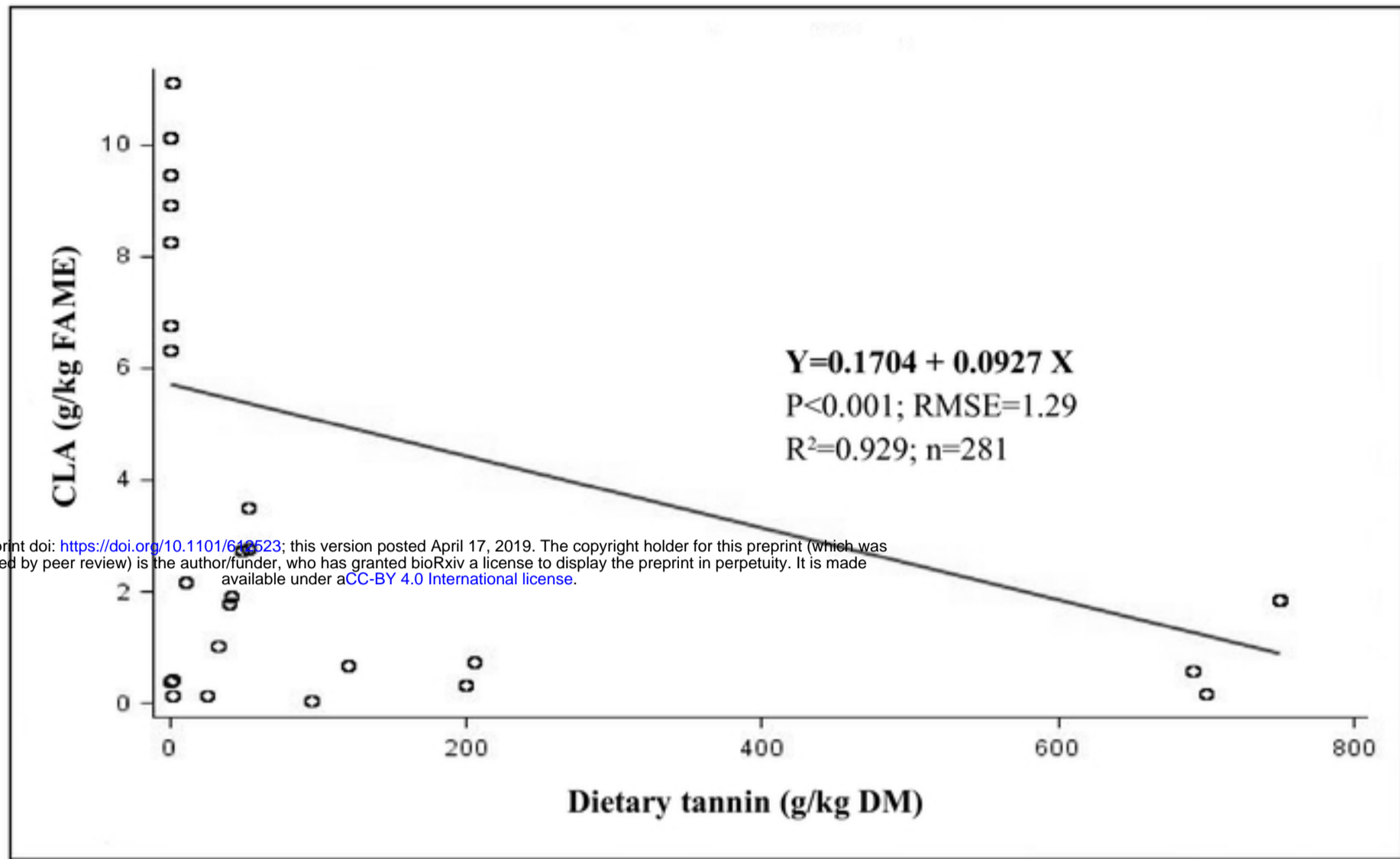


Figure 1

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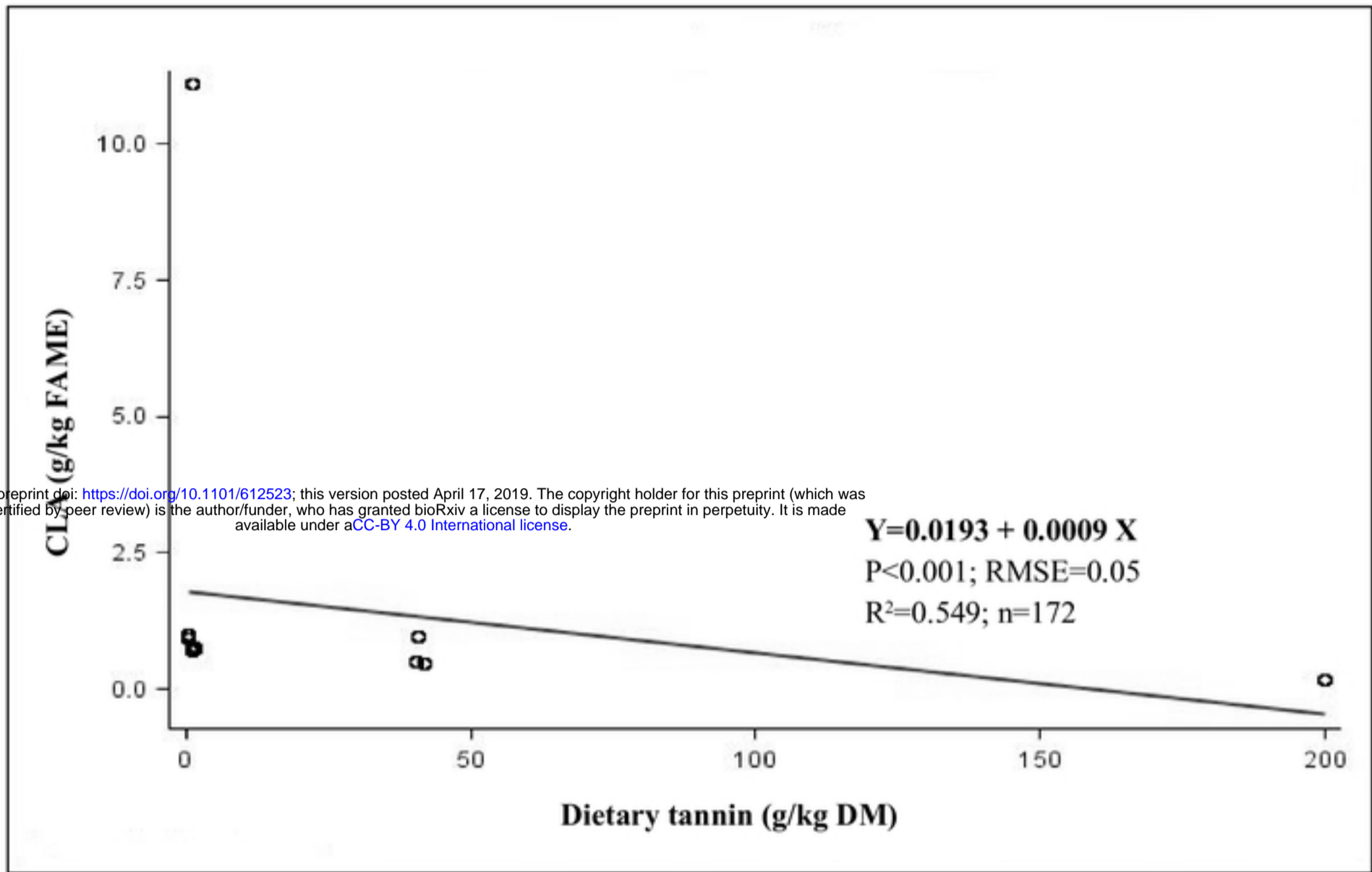


Figure 3

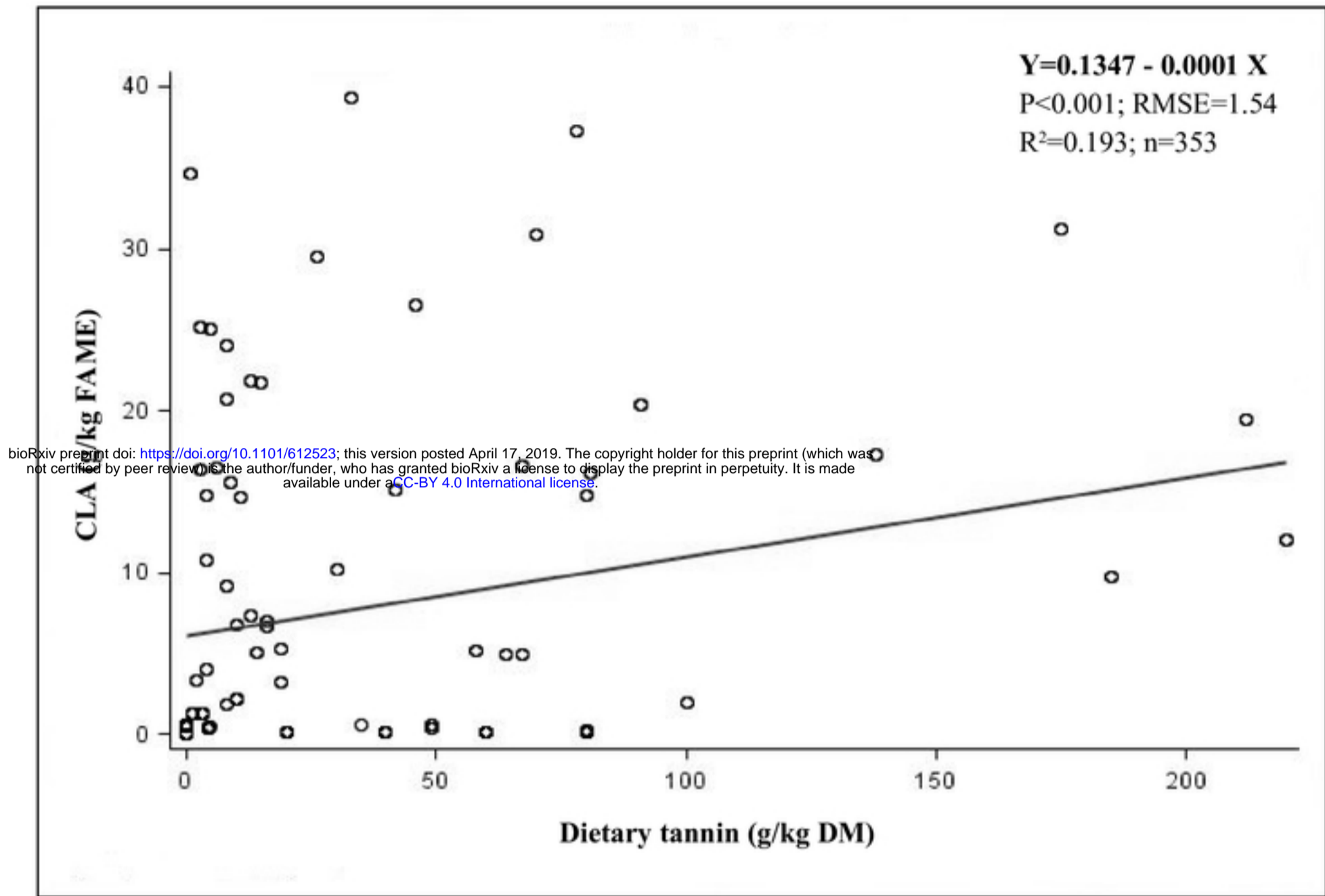


Figure 4

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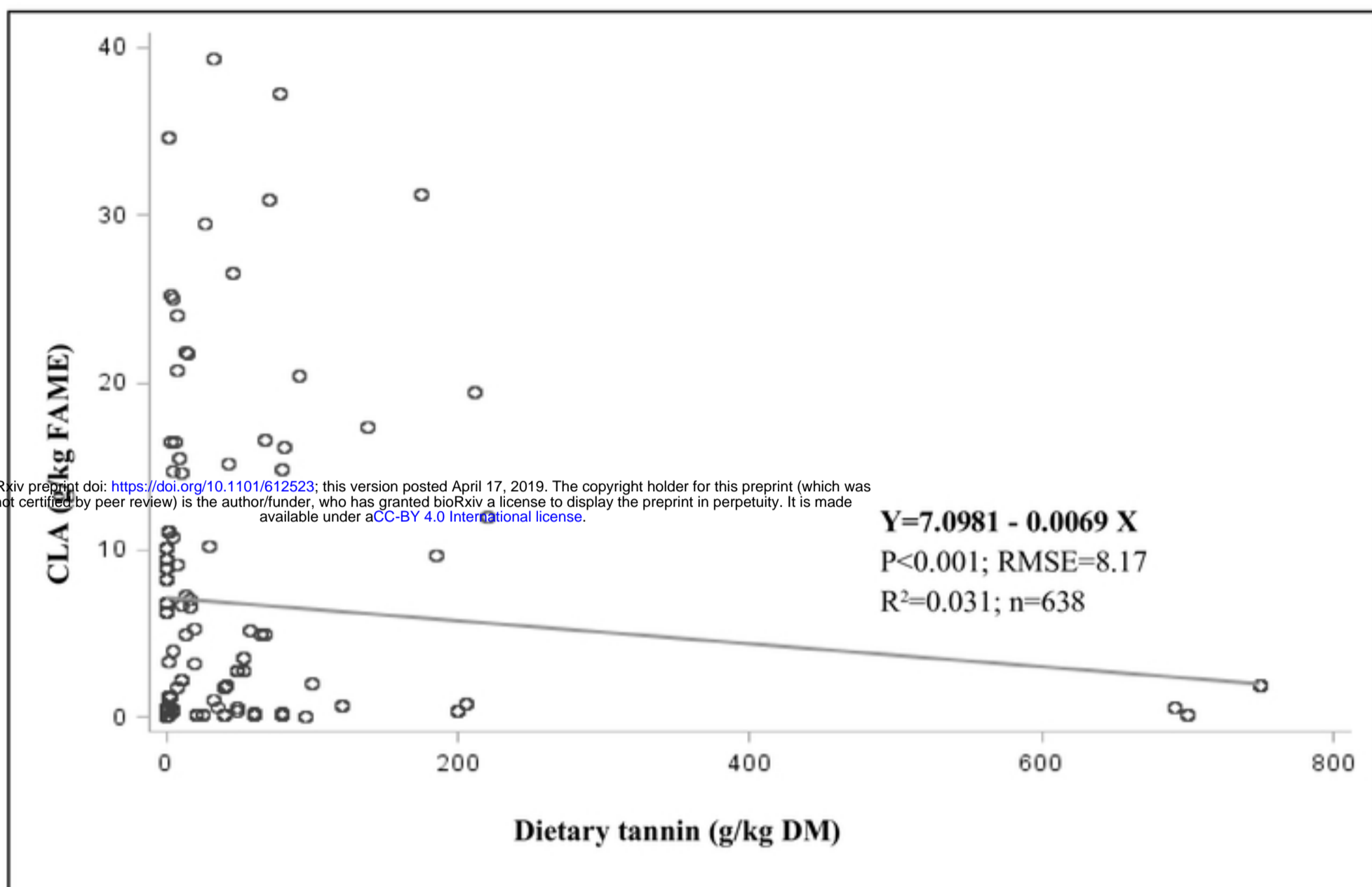


Figure 5

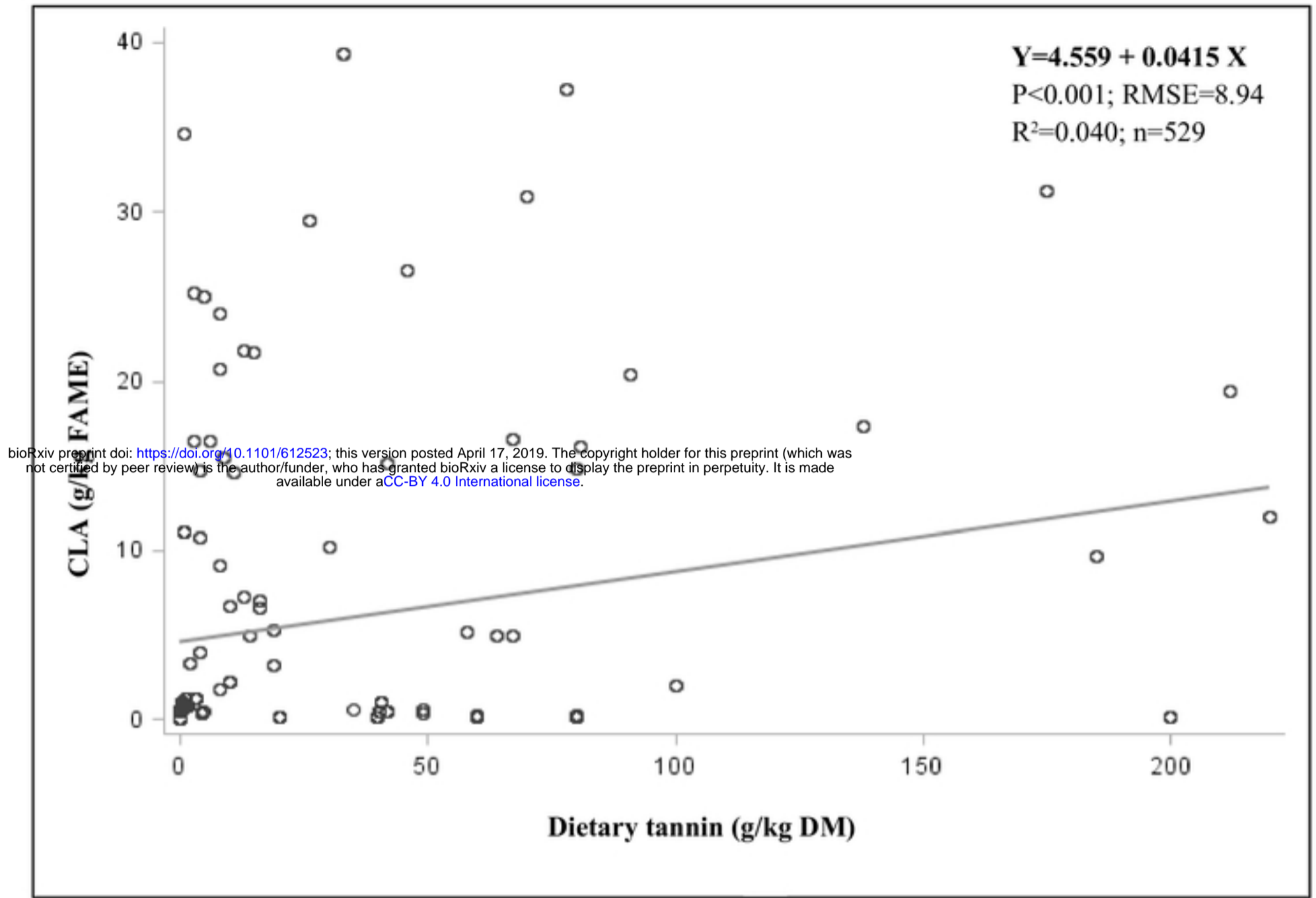


Figure 6