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Effect of dietary black cumin seed (Nigella sativa) on performance, immune status, and serum metabolites of small ruminants: A meta-analysis

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Ms. No. Rumin-D-21-87
Effect of dietary black cumin seed (Nigella sativa) on performance, immune status, and serum metabolites of small ruminants: A meta-analysis
Small Ruminant Research

Dear Mr. Irawan,

I can now inform you that the Editorial Board has evaluated your manuscript. The Editor has advised that the manuscript will be reconsidered for publication after major revision.

The comments listed below should be taken into account when revising the manuscript. Along with your revision, you will need to supply a response

letter ('Revision Note'), which is a thorough, detailed response to the referees' comments, specifically noting each comment made by the

referees and/or Editor, and describing all changes. Should you disagree with any comment(s), please explain why. In case the Associate Editor or a reviewer has supplied a detailed list of small changes please use red type in the text to signal the changes you have made.

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We are looking forward to receiving the revised submission.

With kind regards,

Small Ruminant Research

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Reviewer #1: This paper describes a meta-analysis regarding the effect of black cumin seeds (BCS) at varying levels on performance parameters of goats, lambs and sheep.

The authors have drawn conclusions for these three groups together, without examining the animal effect. I suggest adding the animal effect to the models and then defining study as a random effect nested within animal. Interactions between animal and level could also be considered.

The table summarizing the studies used in the meta-analysis only provides a range of values for %BCS in DM basis. It is not clear whether different values of %BCS appeared in more than one study (except for zero, obviously) nor how many different values appeared in each study. This information should be provided.

L134: Replace a2-hat by beta2-hat.

LL134, 135, 137: Change the subscript of Level from ij to j.

L139 Correct "Statistical models used were …" to "Statistical models were summarized by …".

Tables 2,3,4:
1. Presenting AIC values without comparison to AIC for competing models (such as quadratic versus linear) is meaningless.

2. Rsquared values should be presented for each model.

3. Using a quadratic model instead of a linear model changes the estimates for beta0 and beta1 in addition to adding an estimate for beta2. Therefore the results for the quadratic models should show estimates and standard errors for beta0,, beta1 and beta2, together with p-values for beta1 and beta2.

Reviewer #2: I reviewed the manuscript entitled "Effect of dietary black cumin seed (Nigella sativa) on performance, immune status, and serum metabolites of small ruminants: A meta-analysis." This study aimed to quantify the effect of black cumin seeds (BCS) supplementation on the productive performance, nutrient utilization, and blood metabolites profile of small ruminant animals. In general, I found the topic interesting and relevant to Small Ruminant Research Journal. However...

1. Authors suggested that 23 studies were included in the meta-analysis. However, the number of observations used to develop the equations, in my opinion, is too low to conduct a meta-analysis. Considering that animal feeding assays include several animals to test different statistical. I surprised to see that authors, for

statistical, I surprised to see that authors, for example, developed equations with ¡only six and seven data points! (Table 3) and equations with maximum 37 and 39 data points (Table 2). That sounds weird! My suggestion to authors regarding this issue is trying to get the original data of studies (at least 5 studies) and conduct the meta-analysis with that information. Preferably, explore one species.

2. One fatal flaw of the study in my opinion, is that authors unweighted the studies' observations during the meta-analysis. Authors should consider that they are taking mean observations, and not the original data. According to Sauvant et al., (2008) (a paper cited by the authors): "observations must be weighed to account for differences in the precision of the reported means." Apparently, authors did not conduct this important step, which will result in flawed predictions.

3. Another fatal flaw, in my opinion, is that the species' effect cannot simply be considered within the "study effect". As authors explained, they combined information about "lamb", "sheep", and "goats" in the meta-analysis. Different literature reports suggest that nutrient metabolism differs between these species. Indeed, feeding behavior of sheep and goats is markedly different, and author are exploring "black cumin seeds", a plant seed. Hence, species' effect may influence BCS intake, thereby affecting metabolic response and performance of animals. All these assumptions led to me to think, that species' effect need to be included as an independent effect in the meta-analysis, possibly as random, considering the objective of this study. Another option could be to focus the meta-analysis on only one species.

Other technical issues:

1. English need to be revised. I found many grammar and spelling inconsistencies in several parts of the manuscript.

2. Authors did not provide a table with statistical summary of the dataset used in the meta-analysis.

3. Authors neither mention checks of statistical

principle assumptions nor verification of the presence of influence values or outliers. For example, if the variance is not homogeneous, authors need to use additional statistical tools to overcome this potential limitation (example, variance modelling, Bayesian statistics, etc.). Otherwise, conclusions of your study may be biased.

Reviewer #3: The manuscript was well written, and the results found are relevant to the scientific community. However, I have a few concerns about some approaches that I pointed out in the specific comments that need to be addressed or at least justified if could not be performed.

110. (4)

128-129. 1) Were the variables weighted for variation in precision across studies?
2) Did the authors test the effect of specie? Or at least check for this possible effect? Goats in general have different metabolism on N recycling and adaptation to the environmental from sheep.

197-206. Did the authors check the effect of concentrate level in the diet x BCS inclusion? Or at least to include concentrate level as a covariate in the model?
Did the authors test some covariate to the model? If yeas, you need to include this information in the manuscript. If no, it is necessary to check about it. I mentioned this because it is necessary to "clear" the effect of BCS inclusion in the diet on the animal performance. How was the average experimental diets composition according to the BCS inclusion? It could be more interesting to verify if there is no effect of some diet characteristic across BCS inclusion to allow the author to give all the credit to the BCS inclusion effects on the animal performance.
201-206. why the authors did not check the effect of BCS nutritional characteristics on animal performance instead to make assumptions and speculation.

207-209. Please, show a summary of chemical composition of the diets and BCS. Why the authors did not covariate the model's whit ETHER

EXTRACT contends?

228. Odhaib et al. (2018a) found.

236. Yalcin et al., (2012)

237-241. Confuse. I did not understand the connection of this discussion with you results. Not only blood metabolites are the indicators of health status, because IgG, IgA, and IgM are indicators of immunity which is also health. What the authors mean with "medicinal plants"? And what the authors say about the Total white blood cells in the discussion?
This section about Health status and immunology response need to be improved by the authors.

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Dear Dr. S.Y. Landau

Editor-in-Chief of ***Small Ruminant Research***,

SUBJECT: Re-submission of revised manuscript (**RUMIN-D-21-87-R1)**

First of all, we would like to express our gratitude for the second opportunity to revise our manuscript entitled "**Effect of dietary black cumin seed (Nigella sativa) on performance, immune status, and serum metabolites of small ruminants: A meta-analysis''** for your consideration toward publication in **Small Ruminant Research**.

We acknowledge the time and efforts by the editor and reviewers in reviewing our manuscript.

We have carefully considered and respond all of the comments from reviewers (attached). We made substantial revision as suggested in the revised manuscript, i.e. we included the interaction effects between concentrate and BCS inclusion and included specific animal intercepts in the models. We also provided graphical representation from the models (Figure 1 and Figure 2) to visualize the results.

With the above we trust we have properly addressed all the weaknesses uncovered by the reviewer. Once again, we are very grateful to the reviewer for their constructive suggestions. We hope that you will finally accept our manuscript for publication.

We are looking forward to receiving your further communications.

Yours sincerely,

Mr. Agung Irawan, on behalf of the co-authors

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Responses to Reviewers for Small Ruminant Research

Reviewer #1

We thank the reviewer for the constructive suggestion and evaluation on our manuscript. Herein, we provide our responses point by point as follows:

1. In LL132-139 - please define indices i and a.
   Response: We have defined the i and a indices in the paragraph (L133-143).

2. L137: Surely Xi is the level of BCS in study (sample?) i.
   Response: Xi is the level of BCS in the study a, we have scrutinized our models and provided in details (L135-143).

3. LL145-146: Interaction between dietary concentrate level and BCS inclusion level is not the issue. If dietary concentrate level has a significant effect in the meta-regression analysis then it should be retained as a covariate in the model.
   Response: We have included the interaction effect between concentrate level and BCS level in the model (Table 3, 4, 5).

4. Tables 3, 4 and 5: Estimates of the animal-specific intercepts are missing.
   Response: We have added the animal specific intercepts in the Tables 3, 4, 5, especially for those had significant effect and more than 1 study sources. For those came from only one species, it has also been specified in the Table.

5. From the detailed values of BCS in Table 1 it appears that in all the database 40 levels of BCS were 1.2 or less and the remaining 12 values ranged from 5 to 25, half of them for sheep, half for lambs and none for goats. Therefore the goat effect is confounded with a low level of BCS, and models for all three animals together are not valid. As there were only five studies for goats a possible solution is to omit these studies from the meta-analysis. Some graphics of the main outcome variables from Table 3 versus BCS values, with animal identified on the graph, could help to resolve this problem.
   Response: We really thank for this suggestion. It really helped us to re-evaluate our data and under present form, it looks clear especially for the effect of animal-BCS interaction on DMI.

We have omitted the goat from the data for the performance parameters as suggested. We have also presented graphical visualisations (see Figure 1 and Figure2), following the methods from St-Pierre (2001) using the residual values for adjustment of the response variables (Y value) (L151-152).

With the above we trust we have properly addressed all the weaknesses uncovered by the reviewer. Once again, we are very grateful to the reviewer for the constructive suggestions

1. I suggest adding the animal effect to the models and then defining study as a random effect nested within animal. Interactions between animal and level could also be considered.

   Response:
   Thank you for the advice. We have re-analyzed based on the models proposed by the report reviewers attached to tables 3, 4, and 5. The models used are as follows: The models used are as follows:
   (1) $Y_{ai}=\beta_0+\beta_1 X_i+A_{0a}+\left(A_1 S_0\right)_{ai}+A_3 * X_i+e_{ai}$
   (2) $Y_{ai}=\beta_0+\beta_1 X_i+\beta_1 X_i^2+A_{0a}+\left(A_1 S_0\right)_{ai}+A_3 * X_i+e_{ai}$
   Where the first model (1) is linear mixed model form while the second (2) is a quadratic mixed model. Fixed effects components are $\beta_0+\beta_1 X_i$ (order 1) and $\beta_0+\beta_1 X_i+\beta_1 X_i^2$ (order 2), respectively while the random effects are $A_{0a}+\left(A_1 S_0\right)_{ai}$. Y_{ai} is the dependent variable, β_0 = overall intercept, β_1 = slope for level, X_i = level as value of the continuous outcome variable, $A_{0a}+\left(A_1 S_0\right)_{ai}$ intercept variying among animal and study within animal, $A_3 * X_i$ interaction beetwen animal anad level, and e_{ai} = the unexplained residual error.

2. 197-206. Did the authors check the effect of concentrate level in the diet x BCS inclusion? Or at least to include concentrate level as a covariate in the model?

3. L134: Replace a2-hat by beta2-hat.

   Response:
   Thanks to the reviewer's suggestion of improvement. We have replaced a2-hat with beta2-hat on line 134 $\beta_0+\beta_1 X_i+\beta_1 X_i^2$.

4. LL134, 135, 137: Change the subscript of Level from ij to j.

   Response:
   We have fixed the subscript from level (X) to ij to j and changed the notation from j to i due to the adjustment of the latest model. The replacement does not change its meaning. As in equation number (1).

5. L139 Correct "Statistical models used were …" to "Statistical models were summarized by …".

   Response:
   We have accommodated the reviewer 's direction by correcting the sentence "Statistical models used were…" to "Statistical models were summarized by…" in accordance with the reviewer' s direction.

6. Presenting AIC values without comparison to AIC for competing models (such as quadratic versus linear) is meaningless (Table 2-4)

   Response:

Under the direction of our reviewers have changed the statistical measure AIC with R2 which is attached in Table 3, 4, and 5.

7. Rsquared values should be presented for each model.

   Response:
   R2 of linear and quadratic equations have been added in Table 3, 4, and 5.

8. Using a quadratic model instead of a linear model changes the estimates for beta0 and beta1 in addition to adding an estimate for beta2. Therefore the results for the quadratic models should show estimates and standard errors for beta0,, beta1 and beta2, together with p-values for beta1 and beta2.

   Response:
   In accordance with the advice of reviewer's. We have improved the coefficient of quadratic equations in Table 3, 4, and 5.

9. Authors did not provide a table with statistical summary of the dataset used in the meta-analysis.

   Response:
   We have added a descriptive statistics table from the database in Table 2.

10. Authors neither mention checks of statistical principle assumptions nor verification of the presence of influence values or outliers. For example, if the variance is not homogeneous, authors need to use additional statistical tools to overcome this potential limitation (example, variance modelling, Bayesian statistics, etc.). Otherwise, conclusions of your study may be biased.

    Response:
    As guidelines of the reviewer's, we have added Levene's test to find out the homogeneity of the variants. As shown in tables 3, 4, and 5.

11. 207-209. Please, show a summary of chemical composition of the diets and BCS. Why the authors did not covariate the model's whit ETHER EXTRACT contends?

Table 2. Descriptive statistic of the database

Response variable	Unit	Mean	SD	Max.	Min.
Level of black cumin seed (BCS)	g/Kg of feed as DM Basis	16.9	38.7	200	0
Ration					
Concentrate	%	57.3	17.3	75	24.1
Forage and roughage	%	42.7	16.9	76	25.0
Chemical composition of black cumin seed					
Dry matter	%	91.6	1.08	92.6	88.6
Ash	% DM	3.84	1.48	8.43	2
Organic matter	% DM	96.2	1.48	98	91.6
Crude protein	% DM	23.3	7.07	33.1	7.5
Ether extract	% DM	9.67	2.32	12.7	4.7
Crude fibre	% DM	9.84	4.45	19.9	6.6
Nitrogent free extract	% DM	52.0	11.6	67.9	34.8
Neutral detergen fibre	% DM	42.7	12	55.1	22.8
Acid detergen fibre	% DM	23.7	5.95	29.3	11.4
Hemeicelulose	% DM	19.0	6.33	25.8	11.4
Chemical composition of total mixed ration					
Dry matter	%	92.3	2.28	97.1	89.9
Ash	% DM	8.68	3.26	14.6	5.17
Organic matter	% DM	91.3	3.26	94.8	85.4
Crude protein	% DM	15.2	2.92	22	9.30
Ether extract	% DM	4.29	2.15	9.02	1.90
Crude fibre	% DM	12.1	6.82	21.2	3.07
Nitrogen free extract	% DM	60.6	8.64	70	39.6
Neutral detergen fibre	% DM	42.2	7.36	55.7	29.3
Acid detergen fibre	% DM	17.6	6.44	27.4	6.98
Celulose	% DM	15.5	0.0577	15.5	15.4
Hemeicelulose	% DM	24.6	9.87	39.6	9.30
Lignin	% DM	5.31	1.42	7.31	3.94
Metabolizable energy	Mcal/Kg	2.74	0.313	3.05	2.04
Performance					
Average daily gain (ADG)	g/head/d	153	58.0	272	54.2
Average daily intake (ADi)	g/head/d as DM Basis	1,180	381	2,188	443
Feed convertion ratio (FCR)		7.37	1.92	12.7	3.9
Nutrient digestibility and nitrogen metabolism					
Dry matter	%	67.8	7.71	79.2	56.6

Response variable	Unit	Mean	SD	Max.	Min.
Organic matter	% DM	67.0	7.96	80.3	55.8
Crude protein	% DM	68.9	7.58	78.1	50.6
Ether extract	% DM	65.9	12.2	85.6	39.0
Nitrogen free extract	% DM	71.9	7.98	80.1	62.3
Neutral detergent fibre	% DM	66.3	6.04	72.8	56.8
Acid detergent fibre	% DM	55.1	6.55	60.7	43.8
Nitrogen intake	g/d	20.1	7.69	34.2	11.2
Nitrogen in faces	g/d	5.52	0.98	6.9	4.18
Nitrogen in urine	g/d	5.44	4.17	9.7	0.05
Nitrogen digested	g/d	14.8	6.81	27.3	7.8
Nitrogen retention	g/d	9.33	5.52	19.5	3
Blood metabolites composition					
Albumin	g/dL	3.26	0.653	4.85	2.21
Globulin	g/dL	3.38	1.46	5.89	0.72
Albumin/globulin ratio	g/dL	1.52	1.48	5.55	0.58
Blood urea nitrogen	mg/dL	55.5	31.8	111	12.3
Cholesterol	mg/dL	98.0	55.5	187	29.5
Total protein	g/dL	6.09	1.41	9.85	4.05
Creatinine	mg/dL	0.85	0.62	2.83	0.49
Glucose	mg/dL	59.0	21.1	82.9	17.7
Triglyceride	mg/dL	70.7	48.5	177	12.3
White blood cells (WBC) differentiation					
Total white blood cells	$1x10^3/mm^3$	8.57	2.19	11.4	4.79
Neutrophile	% WBC	51.0	10.6	64.1	36.6
Lymphocyte	% WBC	43.4	6.66	52.0	34.4
Monocyte	% WBC	4.04	1.77	5.4	1.32
Eosinophile	% WBC	1.34	0.49	1.63	0.61
Basophile	% WBC	0.54	0.12	0.67	0.43
Imune response					
Immunoglobulin A	mg/L	446	117	646	269
Immunoglobulin G	g/L	36	18.1	66.9	21.3
Immunoglobulin M	mg/L	162	8	171	156

SD, Standard deviation; Max., Maximum; Min., Minimum.

Table 3. Regression equation of the relationship between dietary levels of black cumin seed (g/kg DM) on performance of small ruminant animals

Parameter outcomes	Unit	M	N	Parameter estimates				Model estimates			Levene's test[2)]	Animal vs Level[3)]
				β0		β1		p-value	RMSE	$R^{2,1)}$		
				Value	SE	Value	SE					
Average daily gain	g/head/d	L	35	120	47	0.22	0.069	0.005	1.07	0.938	0.519	0.69
Average daily intake	g/head/d as DM basis	L	38	889	345	0.618	0.189	0.004	1.2	0.986	0.383	0.386
Feed conversion ratio		L	32	7.42	0.482	-0.0048	0.0044	0.292	1.21	0.797	0.542	0.1

DM, Dry matter; L, Linear; M, Model; N, Number of data; SE, Standard error;

[1)] R^2 = The conditional r-squared value of the mixed effects model based on the Nakagawa method;

[2)] p-value of Levene's test for equality of variances, if p>0.05 the population variances are equal (Fox and Weisberg 2019);

[3)] p-value of interaction beetwen animal and adition level of black cumin seed.

Table 4. Regression equation of the relationship between dietary levels of black cumin seed (g/kg DM) on nutrient digestibility and nitrogen metabolism of small ruminant animals

Parameter outcomes	Unit	M	N	Parameter estimates				Model estimates			Levene's test[2)]	Animal vs Level[3)]
				β0		β1		p-Value	RMSE	$R^{2,1)}$		
				Value	SE	Value	SE					
Dry matter	%	L	17	66.1	2.61	0.066	0.0291	0.0522	1.0315	0.717	0.4	0.85
Organic matter	% DM	L	15	65.5	2.74	0.103	0.0506	0.0812	1.0248	0.774	0.91	0.966
Crude protein	% DM	L	17	67.5	2.37	0.0548	0.025	0.06	1.2030	0.793	0.878	0.931
Ether extract	% DM	L	13	62.7	3.81	0.116	0.0625	0.114	1.1382	0.42	0.721	0.63
Nitrogen free extract	% DM	L	7	70.3	6.26	-0.0258	0.0192	0.272	0.7107	0.978	0.89	0.74
Neutral detergent fiber	% DM	L	10	66.7	3.51	-0.0238	0.0257	0.406	0.8872	0.775	0.656	-
Acid detergent fiber	% DM	L	6	55.6	8.48	-0.019	0.0529	0.745	0.8612	-	0.911	-
N intake	g/d	L	41	17.7	4.46	0.0105	0.0037	0.0097	1.4568	0.99	0.709	0.269
N in feces	g/d	L	9	5.76	0.562	-0.0025	0.0016	0.1964	0.7351	0.921	0.564	-
N in urine	g/d	L	9	6.34	2.08	-0.0051	0.003	0.1648	0.7002	0.983	0.555	-
N digested	g/d	L	9	14.3	3.68	0.0207	0.0034	0.0036	0.8332	0.994	0.641	-
N retention	g/d	Q	9	7.79	2.55	0.0526	0.0029	0.0004	0.5861	0.999	0.753	-
						-0.000149	0.000015	0.0024				

L, Linear; M, Model; N, Number of data; Q, Quadratic; SE, Standard error; TDN, total digestible nutrients

[1)] R^2 = The conditional r-squared value of the mixed effects model based on the Nakagawa method;

[2)] p-value of Levene's test for equality of variances, if p>0.05 the population variances are equal (Fox and Weisberg 2019);

[3)] p-value of interaction beetwen animal and adition level of black cumin seed.

Table 5. Regression equation of the relationship between dietary levels of black cumin seed (g/kg DM) on blood metabolites and immune response of small ruminant animals

Parameter outcomes	Unit	M	N	Parameter estimates				Model estimates			Levene's test[2)]	Animal vs Level[3)]
				β0		β1		p-Value	RMSE	$R^{2,1)}$		
				Value	SE	Value	SE					
Blood metabolites composition												
Albumin	g/dL	L	28	3.27	0.167	0.00195	0.00374	0.609	1.19	0.582	0.375	0.33
Globulin	g/dL	L	20	3.22	0.755	0.0052	0.00408	0.235	0.969	0.927	0.489	0.362
Albumin/globulin ratio	g/dL	L	20	1.67	0.847	-0.000681	0.00721	0.927	1.08	0.743	0.985	0.645
Blood urea nitrogen	mg/dL	L	24	57.4	9.96	0.0447	0.0382	0.265	0.89	0.964	0.865	0.148
Cholesterol	mg/dL	L	19	109	30.5	-0.2	0.0957	0.063	0.862	0.971	0.396	0.303
Total protein	g/dL	L	32	5.93	0.366	0.0319	0.0142	0.04	1.06	0.883	0.188	0.554
Creatinine	mg/dL	L	15	0.851	0.294	0.000574	0.0035	0.874	1.22	0.781	0.974	0.06
Glucose	mg/dL	L	30	56.3	14.7	0.0283	0.0296	0.354	1.03	0.955	0.508	0.643
Triglyceride	mg/dL	L	19	70.1	16.1	0.0068	0.0541	0.902	1.04	0.967	0.661	0.485
White blood cells differentiation												
Total white blood cells	$1x10^3/mm^3$	L	18	7.78	1.29	0.0142	0.0069	0.0696	1.18	0.311	0.6571	0.0033
Neutrophil	% WBC	L	6	51.6	9.25	-0.345	1.16	0.795	0.649	0.789	0.8903	-
Lymphocyte	% WBC	L	6	41.4	6.09	1.11	0.789	0.293	0.624	0.788	0.6752	-
Monocyte	% WBC	L	6	4.29	1.09	-0.14	0.0588	0.14	0.623	0.977	0.8914	-
Eosinophil	% WBC	L	4	1.63	0.642	-0.246	0.176	0.395	0.652	0.396	<0.0001	-
Basophile	% WBC	L	4	0.43	0.048	0.0865	0.013	0.095	0.652	0.936	<0.0001	-
Immune response												
Ig A	mg/L	L	7	387	74.1	2.95	0.617	0.017	0.787	0.922	0.7941	

Parameter outcomes	Unit	M	N	Parameter estimates				Model estimates			Levene's test[2)]	Animal vs Level[3)]
				β0		β1		p-Value	RMSE	$R^{2,1)}$		
				Value	SE	Value	SE					
Ig G	g/L	L	7	28.7	6.32	0.209	0.0242	0.003	0.696	0.981	0.9395	
Ig M	mg/L	L	4	156	1.75	5.47	1.024	0.118	0.652	0.905	<0.001	

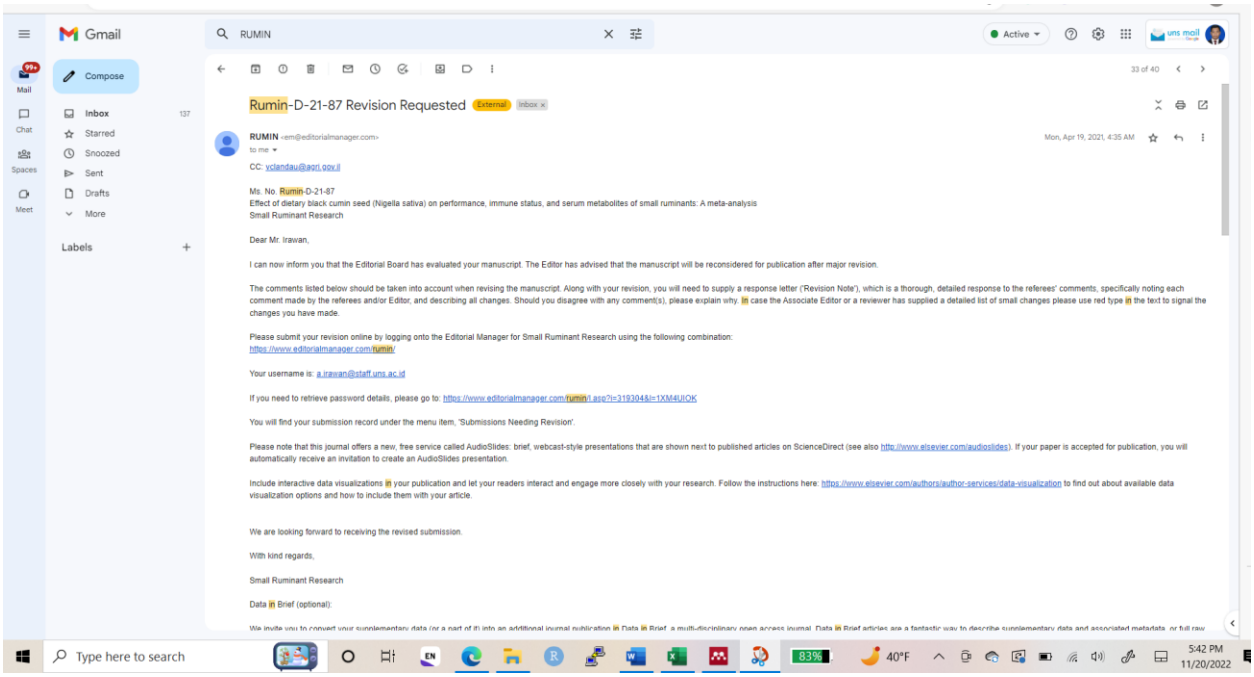
L, Linear; M, Model; N, Number of data; Q, Quadratic; SE, Standard error;

[1)] R^2 = The conditional r-squared value of the mixed effects model based on the Nakagawa method;

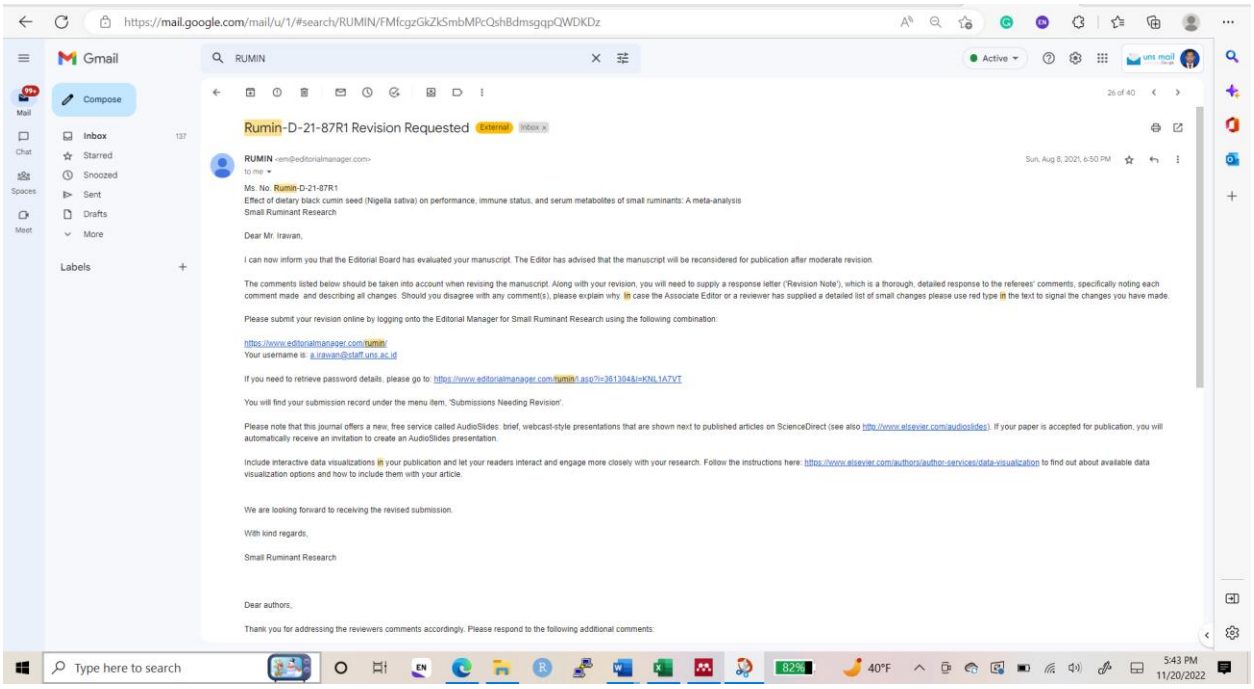
[2)] p-value of Levene's test for equality of variances, if p>0.05 the population variances are equal (Fox and Weisberg 2019);

[3)] p-value of interaction beetwen animal and adition level of black cumin seed.

Editor Decision #1 Major revision



Editor Decision #2 Moderate revision



Editor Decision #3 Accepted