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# Use of yellow mealworm (*Tenebrio molitor*) as a protein source on growth performance, carcass traits, meat quality and intestinal morphology of Japanese quails (*Coturnix japonica*)



Zahra Shariat Zadeh, Farshid Kheiri\*, Mostafa Faghani

Department of Animal Science, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

ARTICLEINFO	A B S T R A C T
<i>Keywords:</i> Fish meal Intestinal morphology Japanese quail Performance Mealworm	This experiment was conducted to examine the effects of <i>Tenebrio molitor</i> (TM) larvae meal inclusion in diets as a replacement for fish meal and soybean oil on growth performance, carcass traits, meat quality, and intestinal morphology of Japanese quails ( <i>Coturnix japonica</i> ). A total of 160 mixed sex quails at seven-day of age were weighed and allocated to 20 cages. The dietary treatments were as follows: control (C) group containing 370 g soybean meal (SBM)/kg of diet and 30 g fish meal (FM)/kg of diet and four <i>T. molitor</i> (TM) larvae meal groups, in which TM meal was included as a replacement for FM and soybean oil at 7.5 (7.5), 15 (TM15), 22.5 (TM22.5) and 30 (TM30) g TM/kg of diet. The use of TM at the levels of 22.5 and 30 g/kg of diet significantly ( $P < 0.05$ ) increased body weight (BW) of the birds compared with other groups. Quails fed 22.5 and 30 g TM/kg of diet had better FCR values compared with other groups. The carcass and breast yields obtained in birds fed 30 g TM/kg of diet was significantly ( $P < 0.05$ ) higher than other groups. Significant increases in villous height and crypt depth in TM supplemented birds was found ( $P < 0.05$ ). In conclusion, our data indicated that increasing TM inclusion up to 30 g/kg of feed in quail diets could improve BW, FCR, carcass yield, meat quality, and histology of iejunum.

# 1. Introduction

The global demand for poultry meat is expected to accelerate because of increasing population, accelerating economic growth and rising health issues. The growth period of quail life is an important phase in realizing the long-term great performance (Elnesr, Ropy, & Abdel-Razik, 2019). Protein sources represent the largest component of poultry feed. Fish meal has been used as an animal protein source in poultry nutrition, because of containing high quantity, quality and digestible protein. Since the fish meal containing essential amino acids, especially sulfur-containing amino acids and lysine; it has a high biological value in poultry nutrition. However, apart from its advantages as the most important conventional animal protein source, but its production availability and cost are major problems about it (FAO, 2013). Thus, efforts have been made to find a replacement for fish meal without any adverse effects on performance of poultry.

Nowadays, edible insects are taking into consideration as a highly nutritious and healthy food source with high protein content, highly unsaturated fats, particularly linoleic and linolenic acids, vitamin, fiber, and mineral contents (Marono et al., 2015; Van Huis et al., 2014). In the European Union, insect meals have been currently supplemented to farm fish and pet animals as a protein sources. Recently, several trials have investigated the efficacy of silkworms (*Bombyx mori* L.) (Ijaiya & Eko, 2009), houseflies (*Musca domestica* L.) (Hwangbo et al., 2009), mealworms (*Tenebrio molitor* L.) (Ballitoc & Sun, 2013; Biasato et al., 2016; Bovera et al., 2015, 2016), and black soldier flies (*Hermetia illucens* L.) (Cullere et al., 2016) as replacements for fish or soybean meal in poultry feeding. Biasato et al. (2017) reported that inclusion of dietary *T. molitor* L. (TM) meal as a replacement for soybean meal in broiler chickens diet could improve body weight (BW), feed intake, carcass traits and erythrocyte counts. Biasato et al. (2018) reported that increasing levels of TM inclusion in broiler chickens diets negatively affect feed conversion ratio (FCR) and intestinal morphology.

Despite widely administration of TM larvae meal in poultry diet, to date no meat quality tests have been conducted; thus the present trial carried out to determine the effects of TM larvae meal inclusion in diets as a replacement for fish meal and soybean oil on growth performance, carcass traits, meat quality, and intestinal morphology of Japanese

\* Corresponding author.

E-mail address: kheiri.f@iaushk.ac.ir (F. Kheiri).

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#### Table 1

Ingredients and calculated content of dietary treatments.

Item	Control	TM7.5	TM15	TM22.5	TM30
Ingredients, g/kg (as-fed)					
Corn (8% CP)	548.2	546.7	545	543.4	541.7
Soybean meal (44% CP)	370	373	379	382	385
Fish meal (63.9% CP)	30.0	22.5	15.0	7.5	0.0
TM meal (46.4% CP)	0.0	7.5	15.5	22.5	30.0
Soybean oil	23.8	21.3	18.8	16.3	13.9
DL-methionine	0.2	0.2	0.3	0.4	0.4
L-lysine	0.9	0.9	0.9	0.9	0.9
L-threonine	1.2	1.2	1.3	1.3	1.3
Choline chloride	1.8	1.8	1.8	1.8	1.8
Di calcium phosphate (22% Ca,	8.0	8.4	8.8	9.2	9.6
17% P)					
Calcium carbonate	11.0	11.4	11.8	12.2	12.7
Sodium chloride	0.9	1.1	1.3	1.5	1.7
Sodium bicarbonate	2	2	2	2	2
Trace mineral premix <sup>a</sup>	1	1	1	1	1
Vitamin premix <sup>b</sup>	1	1	1	1	1
Calculated composition, g/kg					
Metabolizable energy, kcal/kg	2900	2900	2900	2900	2900
Crude protein	240	240	240	240	240
Lysine	13	13	13	13	13
Methionine	3.7	3.7	3.8	3.8	3.8
Methionine + cysteine	7.5	7.5	7.5	7.5	7.5
Threonine	10.2	10.2	10.2	10.2	10.2
Tryptophan	2.7	2.7	2.7	2.8	2.8
Arginine	15.1	15.1	15.1	15.1	15.1
Valine	11.3	11.3	11.3	11.3	11.3
Isoleucine	10.3	10.3	10.3	10.3	10.3
Calcium	8	8	8	8	8
Available P	3	3	3	3	3
Analyzed content, g/kg					
Crude protein	242	238	241	243	239

TM, Tenebrio molitor.

<sup>a</sup> Provided the following per kilogram of diet: Mg, 60 mg; Fe, 120 mg; Cu, 5 mg; Zn, 25 mg; Se, 0.2 mg; I, 0.3 mg.

<sup>b</sup> Provided the following per kilogram of diet: vitamin A, 1650 IU; vitamin D<sub>3</sub>, 750 IU; vitamin E,12 IU; vitamin K, 1 mg; riboflavin, 4 mg; vitamin B<sub>12</sub>, 0.003 mg; pantothenic acid, 10 mg; nicotinic acid, 40 mg; folic acid, 1 mg.

quails (Coturnix japonica).

# 2. Materials and methods

#### 2.1. Animals and dietary treatments

A total of 160 mixed sex broiler quails (C. japonica) at seven-day of age were individually weighed and randomly allocated to five treatment groups, each with 4 replicate pens of 8 chicks (average weight:  $19 \pm 1$  g). The dietary treatments were as follows: a control (C) group containing 370 g soybean meal (SBM)/kg of diet and 30 g fish meal (FM)/kg of diet and four T. molitor (TM) larvae meal groups, in which TM meal was included as a replacement for FM and soybean oil at 7.5 (TM7.5), 15 (TM15), 22.5 (TM22.5) and 30 (TM30) g/kg of diet, respectively. Table 1 shows dietary treatments formulated to adequately provide nutritional requirements (National Research Council, 1994) of Japanese quails. All dietary treatments were isocaloric and isonitrogenous and were formulated using the apparent metabolizable energy corrected for N retention values for TM assayed in vivo for broiler quails (unpublished data) according to the method described by De Marco et al. (2015). The bird house temperature was controlled by central heating and was gradually reduced, 33-28 °C in the second week, and 27-22 °C in the third week, and finally maintained at 20 °C. Feed and water were provided ad libitum. Lighting schedule was a period of 23 h light: 1 h darkness.

# 2.2. Analysis of yellow mealworm content

Prior to formulating the diets, corn, soybean meal, FM and TM were analyzed for crude protein (Method 990.03; AOAC, 2006), total amino acids contents (Methods 982.30E a, b, and c; AOAC, 2006), ether extract (Method 920.39A; AOAC, 2006). Calcium (Ca) and total P (tP) of TM were determined by Inductively coupled plasma - optical emission spectrometry (Method 2011.14; AOAC, 1990) at the Islamic Azad University of Shahrekord Laboratories (Islamic Azad University of Shahrekord, Iran).

#### 2.3. Performance and carcass components

Average daily weight gain (DWG), and average daily feed intake (DFI), were recorded in the whole trial, mortality was recorded daily to correct DFI, and DFI: DWG (FCR) was calculated accordingly.

At 35 d of age, after a 2-h feed deprivation 2 male birds per replicate were chosen based on the average weight of the pens, weighed and sacrificed by standard method (Gheisari, Shahrvand, & Landy, 2017; Landy, Ghalamkari, & Toghyani, 2011). Carcass weights, pectoralis major muscle, legs and internal organ weights (Liver, heart, proventriculus and gizzard) were weighed and expressed as percentage of live body weight.

# 2.4. Morphology of the jejunum

At 35 d of age 2 males per replicate (8 birds per treatment) were killed and one cm segment of the midpoint of the jejunum was separated, rinsed with phosphate buffered saline and fixed and immersed in formalin. The samples were processed, paraffin embedded and two samples of each section (6  $\mu$ m thicknesses) were taken and stained with hematoxylin and eosin, and morphology of the jejunum was evaluated thereafter using light microscopy (Nikon Eclipse 80i, Nikon Co., Tokyo, Japan) according to the method of Kavyani et al. (2014).

# 2.5. Physical and sensory characteristics of breast muscle

At 35 d of age, 2 male birds/replicate were killed, boneless breasts without skin from left side of sternum were separated. Samples from three parts of the muscles were frozen for 24 h, and color was defined using a Chroma Meter CR-310 colorimeter (Minolta, Osaka, Japan). Water retention capacity was determined according to the method described by Delezie, Swennen, Buyse, and Decuypere (2007). Cooked samples were weighed, calculated as a percentage of samples before freezing, and expressed as cook loss.

# 2.6. Statistical analysis

Data were subjected to analysis of variance procedures appropriate for a completely randomized design and analyzed by one-way ANOVA using the General Linear Model procedures of SAS (SAS Inst. Inc., Cary, NC). Means were compared using Tukey's HSD multiple range test. Statements of statistical significance are based on P < 0.05.

# 3. Results and discussion

# 3.1. Performance and carcass traits

Data about TM analysis for crude protein, total amino acids, Ca and tP contents are presented in Table 2. The use of TM as a replacement for FM at the levels of 22.5 (269.6 g) and 30 (260.9 g) g/kg of diet significantly (P < 0.05) increased final BW of the birds compared with quails fed the basal diet (245.1 g) or a basal diet supplemented with 7.5 (234.0 g) or 15 (236.8 g) g TM/kg of diet. DFI of quails was significantly (P < 0.05) higher in birds fed the basal diet (18.89 g/d) compared with birds fed 7.5 (17.04 g/d), 15 (17.79 g/d), 22.5 (17.28 g/d) and 30

#### Table 2

|--|

Composition of mealworm larvae (Tenebrio n	nolitor), g/kg
Total protein ( $N \times 6.25$ )	46.44
Arg	22.28
His	13.79
Ile	18.29
Leu	31.28
Lys	25.04
Met	5.22
Cys	6.67
Phe	15.44
Thr	17.03
Val	25.74
Calcium	0.43
Total phosphorus	7.06

# Table 3

Effect of the dietary mealworm larvae supplementation on the growth performance of the Japanese quails (*Coturnix japonica*).<sup>a</sup>

Variable	Dietary t Control	reatments TM7.5	TM15	TM22.5	TM30	SEM
Body weight, g	$245.1^{b}$	234.0 <sup>c</sup>	236.8 <sup>c</sup>	$269.6^{a}$	260.9 <sup>a</sup>	4.89
Daily feed intake, g/d	$18.89^{a}$	17.04 <sup>b</sup>	17.79 <sup>b</sup>	$17.28^{b}$	16.34 <sup>c</sup>	0.62
Daily weight gain, g/d	$8.09^{b}$	7.68 <sup>c</sup>	7.78 <sup>c</sup>	$8.95^{a}$	8.64 <sup>a</sup>	0.37
FCR, g:g	$2.34^{a}$	2.22 <sup>b</sup>	2.29 <sup>b</sup>	$1.93^{c}$	1.90 <sup>d</sup>	0.02

TM, Tenebrio molitor; FCR = feed:gain ratio; SEM = standard error of mean.

<sup>a-d</sup> values in the same row not sharing a common superscript differ (P < 0.05).

<sup>a</sup> Data are means of 4 replicate cages consisting of 8 birds per replicate cage.

(16.34 g/d) g TM/kg of diet as a replacement for FM. Significant differences between treatments were noted in FCR (P < 0.05) as quails fed 22.5 (1.93) and 30 (1.90) g TM/kg of diet had better FCR values compared with growing quails fed the basal diet (2.34), or a basal diet supplemented with 7.5 (2.22) or 15 (2.29) g TM/kg of diet.

In the present trial the final BW, DWG and FCR of the birds were improved with increasing TM meal inclusion rate up to 30 g/kg of feed, but the DFI was decreased. These results are consistent with those reported by Ballitoc and Sun (2013) and Bovera et al. (2015) who reported positive effects of TM meal supplementation in fast-growing chicken's diet. Similarly, Biasato et al. (2017) reported an enhancement in BW, weight gain and feed intake of broiler chickens offered diets containing TM meal in comparison with those fed the basal diet. However, Biasato et al. (2017) reported that supplementing broiler chickens diet with TM meal could improve BW and DFI, but negatively affect FCR. Similarly, Loponte et al. (2017) reported that the addition of 250 to 500 g TM meal/kg of diet in partridges improved growth performance. In contrast with the results obtained in this study Ramos-Elorduy, Gonzàlez, Hernàndez, and Pino (2002) and Biasato et al. (2016) reported that addition of TM meal in the range of 50 to 100 g/kg of diet did not affect growth performance of fast-growing and intermediate-growing chickens, respectively. In this regard, it has been reported that nutritive value of the insect meal may be affected by the species, life stage, and the substrate which have been used for insect rearing, thus the wide variability of the results may be due to nutritive value of the insect meal (Marono et al., 2015; Sánchez-Muros, Barroso, & Manzano-Agugliaro, 2014). In the current trial the DFI of quails was decreased by increasing levels of TM meal inclusion. The detrimental effect of TM meal on DFI of quails may be induced by the chitin content of TM meal which can decrease the palatability of the feed (Bovera et al., 2015).

In the present study the growing quails fed 22.5 and 30 g TM/kg of diet had better FCR values compared with other groups. As indicated in Table 5, an increase in jejunum villous height and crypt depth in TM meal fed growing quails was found. Since increments in gut

#### Table 4

Effect	of	dietary	mealworm	larvae	supplementation	on	carcass	traits	of
Japane	ese	quails at	35 d of age	a					

Variable	Dietary tr Control	reatments TM7.5	TM15	TM22.5	TM30	SEM
Carcass yield, % Breas yield, % Legs yield, % Liver, % Heart, % Proventriculus, % Gizzard, %	$71.78^{a}$ $13.92^{c}$ $6.55^{ab}$ $1.28$ $0.18$ $0.23$ $1.14$	$67.42^{b}$ $15.92^{b}$ $6.23^{b}$ 1.30 0.23 0.26 1.15	64.47 <sup>c</sup> 13.97 <sup>c</sup> 7.21 <sup>a</sup> 1.33 0.23 0.27 1.13	$62.24^{c} \\ 15.79^{b} \\ 6.76^{ab} \\ 1.28 \\ 0.22 \\ 0.25 \\ 1.14$	72.67 <sup>a</sup> 16.62 <sup>a</sup> 7.44 <sup>a</sup> 1.28 0.17 0.25 1.16	0.72 0.022 0.095 0.048 0.040 0.053 0.056

TM, Tenebrio molitor; SEM = standard error of mean.

<sup>a-e</sup> values in the same row not sharing a common superscript differ (P < 0.05). <sup>a</sup> Data are means of 4 replicate cages with 2 birds/pen.

development such as higher villi length and deeper crypts can lead to more absorption of nutrients thus improved FCR obtained in this study may be associated with more development of gut and thus better nutrients absorption (Kavyani et al., 2014).

Table 4 shows carcass, pectoralis major muscle, and internal organ weight as a percentage of live body weight at 35 d of age. The carcass yield obtained in birds fed 30 g TM/kg of diet (72.67%) was significantly (P < 0.05) higher than those fed TM as a replacement for FM at the levels of 7.5 (67.42%), 15 (64.47%) and 22.5 (62.24%) but did not differ from the birds fed the basal diet (71.78%). Similarly, the percentage of pectoralis major muscle obtained in birds fed 30 g TM/kg of diet (16.62%) was significantly (P < 0.05) higher than those fed the basal diet (13.92%) or diets supplemented with 22.5 (15.79%), 15 (13.97%), 7.5 (15.92%) g TM/kg of diet. The use of TM as a replacement for FM at the levels of 30 (7.44%) and 15 (7.21%) g/kg of diet significantly (P < 0.05) increased legs yield of the birds compared with growing quails fed TM at the level of 7.5 (6.23%) g/kg of diet, but did not differ from those fed the basal diet (6.55%) or a basal diet containing 22.5 (6.76%) g TM/kg of diet. The relative weight of liver, heart, gizzard and proventriculus were not markedly affected by dietary treatments. These findings are consistent with the results obtained by Hwangbo et al. (2009) and Ballitoc and Sun (2013); Khatun, Howlider, Rahman, and Hasanuzzaman (2003), who reported that inclusion of insect meal in broiler chickens diet improved slaughter, dressed carcass, breast muscle and thigh muscle weights and dressing percentage. Islam and Chul-Ju (2017) reported that supplementation of TM and super mealworm (Zophobas morio) as alternatives to antibiotics in broiler chicks diets challenged with Salmonella and Escherichia coli could not affect relative weight of internal organ except for the relative weight of bursa which was decreased by insect meal supplementation. As reported by Shokraneh, Ghalamkari, Toghyani, and Landy (2016) the function of immune system is related to development of lymphoid organs, and to date no immune system investigation have been conducted; so further researches are needed to investigate the effects of TM supplementation on immune system function. Similarly, Biasato et al. (2017) reported that the percentage of liver and gizzard were not affected in broiler chickens fed different levels of TM meal. In contrast with the results obtained in the present trial, Ballitoc and Sun (2013) reported that supplementation of TM to broiler chickens diet increased relative weight of gizzard compared to the control.

# 3.2. Morphometric analysis of the jejunum

Table 5 shows the effects of mealworm larvae supplementation on villus height (VH) and width (VW), crypt depth (CD), and epithelial thickness (ET) in jejunum of male birds at d 35 of age. The ET was lower in birds fed the basal diet or diets containing 30 g TM/kg of diet than other groups (P < 0.05). The VW was significantly (P < 0.05) lower in birds fed diets containing 30 g TM/kg of diet (6.8 µm) than those fed the

# Table 5

Effects of mealworm larvae supplementation on villus height and width, crypt depth, and epithelial thickness in jejunum at d 35 of age.<sup>a</sup>

Variable	Dietary t Control	reatments TM7.5	TM15	TM22.5	TM30	SEM
Villus height (µm)	30 <sup>c</sup>	32 <sup>bc</sup>	31 <sup>bc</sup>	$33^{ab}$	36 <sup>a</sup>	0.68
Crypt depth (µm)	6 <sup>b</sup>	6 <sup>b</sup>	7 <sup>ab</sup>	$7^{ab}$	9 <sup>a</sup>	0.50
Villus width (µm)	10.0 <sup>a</sup>	9.0 <sup>a</sup>	8.5 <sup>ab</sup>	$8.3^{ab}$	6.8 <sup>b</sup>	0.08
Epithelial thickness (µm)	10 <sup>b</sup>	20 <sup>a</sup>	20 <sup>a</sup>	$20^{a}$	10 <sup>b</sup>	3.5

TM, Tenebrio molitor; SEM = standard error of mean.

<sup>a-c</sup> values in the same row not sharing a common superscript differ (P < 0.05). <sup>a</sup> Data are means of 4 replicate cages with 2 birds/pen.

#### Table 6

Effects of mealworm larvae supplementation on physical and sensory characteristics of the breast meat at d 35 of age.  $^{\rm a}$ 

Variable	Dietary tro Control	eatments TM7.5	TM15	TM22.5	TM30	SEM
WRC (%)	$23.24^{ab} \\ 29.06 \\ 43.35 \\ 7.27^{b} \\ 12.95^{a}$	23.78 <sup>ab</sup>	$23.89^{ab}$	$22.78^{b}$	25.72 <sup>a</sup>	0.63
CL (%)		29.08	27.03	29.06	27.37	0.56
L*		43.41	43.23	43.16	43.09	0.09
a*		7.23 <sup>b</sup>	7.19 <sup>b</sup>	7.25 <sup>b</sup>	7.63 <sup>a</sup>	0.03
b*		12.23 <sup>b</sup>	12.21 <sup>b</sup>	12.11 <sup>b</sup>	12.13 <sup>b</sup>	0.11

WRC: water retention capacity; CL: cook loss; L\*: lightness; a\*: redness; b\*: yellowness; TM, *Tenebrio molitor*; SEM = standard error of mean.

<sup>a-b</sup> values in the same row not sharing a common superscript differ (P < 0.05). <sup>a</sup> Data are means of 4 replicate cages with 2 birds/pen.

basal diet (10.0  $\mu$ m), and a basal diet supplemented with 7.5 g TM/kg of diet (8.0 µm) but didn't differ from those fed diets supplemented with 15 (8.5  $\mu$ m), and 22.5 (8.3  $\mu$ m) g TM/kg of diet. Growing quails fed 22.5 and 30 g TM/kg of diet had significantly (P < 0.05) higher VH compared with other groups. Growing quails fed diets containing 30 g TM/ kg of diet (9 µm) had significantly higher CD compared with those fed the basal diet (6  $\mu$ m), or a basal diet supplemented with 7.5 g TM/kg of diet (6 µm), but did not significantly differ from those fed diets containing 15 (7  $\mu$ m) or 22.5 (7  $\mu$ m) g TM/kg of diet (P > 0.05). Biasato et al. (2017) reported that TM meal inclusion did not influence the gut morphology of the broiler chickens. Biasato et al. (2018) investigated efficacy of different levels of TM meal as a replacement for soybean meal and oil (0, 50, 100 and 150 g TM/kg of diet). The results indicated that increasing levels of dietary TM meal inclusion negatively affect feed efficiency and intestinal morphology of broiler chickens. The contrary of obtained results in the present study and other experiments (Biasato et al., 2016, 2017, 2018) may be due to the lower levels which we have used in our experiment.

# 3.3. Physical and sensory characteristics

Table 6 shows the effects of mealworm larvae supplementation on physical and sensory characteristics of breast muscle. Water retention capacity was increased by enhancing the level of TM supplementation (P < 0.05). Treatments failed to induce marked effects on cooking loss, although it tended to improve in growing quails supplemented with 15 or 30 g TM/kg of diet (P > 0.05). Lightness was not affected by the dietary treatments. Samples from growing quails fed diets containing 30 g TM/kg of diet had significantly (P < 0.05) higher redness compared with other groups. Yellowness was significantly (P < 0.05) decreased by increasing the level of TM supplementation. Onsongo et al. (2018) reported that supplementation of black soldier fly larvae meal to broiler chickens diet had no effects on aroma, taste, and overall acceptability of cooked breast meat. Similarly, Sealey et al. (2011) reported that supplementation of black soldier fly larvae meal to fish diet up to 50% did not induce any effects on meat sensory. Cullere, Schiavone, Dabbou, Gasco, and Dalle Zotte (2019) reported

that supplementation of black soldier fly larvae fat as an alternative fat source in broiler chickens diet did not induce any effects on leg weight, thawing and cooking losses, pH, and color values.

#### 4. Conclusion

In conclusion, the present experiment indicated that increasing levels of dietary TM in growing quails diets up to 30 g/kg could improve final BW and FCR. It also could improve carcass yield, meat quality, and morphology of jejunum. These data confirm previous data about TM administration in broilers.

# Ethical approval

The birds were raised in accordance with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals. Ethical approval for the experiment was received from Animal Ethics Committee of the Faculty of Animal Science, Islamic Azad University of Shahrekord (approval ref. no. 2017-056).

# **Declaration of Competing Interest**

We declare that we have no conflict of interest.

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