J I T A A
Journal of the Indonesian Tropical Animal Agriculture
Accredited by Ditjen Riset, Teknologi dan Pengabdian kepada Masyarakat No. 164/E/KPT/2021

J. Indonesian Trop. Anim. Agric.
pISSN 2087-8273 eISSN 2460-6278
http://ejournal.undip.ac.id/index.php/jitaa
47(2):87-96, June 2022
DOI: 10.14710/jitaa.47.2. 87-96

Effects of yellow mealworm larvae (*Tenebrio molitor*) and turmeric powder (curcuma) on laying hens performance, physical and nutritional eggs quality

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Received July 15, 2021; Accepted November 19, 2021

ABSTRACT

Soybean meal is the main source of protein in laying hens' diet that is generally imported. This study aims to explore the yellow mealworm larvae (*Tenebrio molitor*) "TM", and curcuma effects on laying hens' performances, and physical and nutritional eggs quality. One hundred laying hens were divided randomly into 4 groups. The first group (Control) received a standard commercial diet (SCD), while test groups (TM, TP, and TM-TP) received SCD with respectively 5% TM, 0.50 % curcuma, and 5% TM and 0.50% curcuma. According to the results, Control and TM reduced significantly hen's bodyweight in the experiment end. However, the curcuma incorporation and TM (TP and TM-TP) kept the stability of bodyweight during the whole period. No effect of diet was observed on feed intake and egg laying rate. Diet had a significant effect on eggs physical parameters (weight, freshness, thickness and rupture force). The mixture of TM and curcuma permitted eggs with good physical parameters. For nutritional quality of eggs, all groups had the same content of ALA and DHA. However, eggs of group receiving a mixture of TM and curcuma had the lowest cholesterol content. Thus, TM and curcuma could reduce soybean importation dependence with improving eggs quality.

Keywords: egg quality, laying hens, laying performance, turmeric powder, yellow mealworm larvae.

INTRODUCTION

The Soybean is at the core of the modern animal feed system. It is as important as cereals in feed rations for livestock, because it is rich in proteins and amino acids. Beside animal meal banned in some countries since 2000's (Baeten *et al.*, 2005), no other protein source achieves such technical inclusion power, which provides important growth and productivity. Soy meal has nutritional qualities, which make of it a main animal feed component in the intensive farming because it contains about 42 to 48% of a total nitrogenous matter. It is also considered as a good complement of cereals, which are poor in proteins, especially in lysine, an essential amino acid, very abundant in soybean meal. Finally, it has a good digestibility for all animal species (Laudadio and Tufarelli, 2012).

In Algeria, soybean meal is the main source of proteins used in animal feed, in particular for monogastric (Ait Kaki *et al.,* 2018; Moula *et al.,* 2019). Reducing the dependence on imported soybean, which its price is volatile (Laudadio and Tufarelli, 2010), is important for the poultry sector because the animal feed corresponds to more than a half of the production cost (Van Horne, 2019). Moreover, soybean is often produced from genetically modified organisms (GMOs) which are perceived rather negatively by the consumer (Laudadio and Tufarelli, 2012). They are also not authorized in organic farming and the additional cost linked to the use of non-GMO soybean meal could reach $ 150 per ton.

Recently, the use of insects as an animal feed has become more prevalent. This is due to their high content in proteins, unsaturated fats, particularly linoleic and linolenic acids, vitamins, fibers and minerals such as copper, iron, magnesium, manganese, phosphorus, selenium and zinc (FAO, 2013; Marono *et al.,* 2015; Moula and Detilleux, 2019). The insects having a highest potential for a large-scale production to be used in diet of poultry, are the black soldier fly (*Hermetia illucens*), common housefly (*Musca domestica*), and yellow mealworm (*Tenebrio molitor;* TM) (Veldkamp and Bosch, 2015). There are many studies which supplemented insect meal in replace of fish meal or soybean meal in diet of broiler chickens (Biasato *et al.,* 2018) and laying hens (Marono *et al.,* 2017; Bovera *et al.,* 2018).

Insects are considered as a useful alternative to soybean and fishmeal in animal nutrition (Henry *et al.,* 2015; Gasco *et al.,* 2016). Certain insect species are efficient feed converters because they are poikilotherms, so they did not need energy to keep their body temperature constant (Sánchez-Muros *et al.,* 2014). In fact, on average, 2 kg of feed is needed to produce 1 kg of insects, while cattle need about 8 kg (FAO, 2013). In addition, insects use much less water and are less dependent on soil, consequently, less greenhouse effect gas and pollution. Furthermore, insects could feed on organic waste (food and human waste, compost) and transform all this to high protein quality that could be used for animal feed. Finally, all insect forms (adult, larval and pupal forms) are naturally consumed by wild birds and chickens (Zuidhof *et al.,* 2003; FAO, 2013).

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione; diferuloylmethane), extracted from turmeric roots, is a major bioactive polyphenol, considered as a pharmacologically safe agent with anti-oxidant, anti-inflammatory, lipid-lowering, cancer chemo-preventive, and chemotherapeutic potential properties (Esatbeyoglu *et al.,* 2012). However, the research on its effects on the animal growth and health is still in its one's years. It has been established that curcumin ameliorates heat stress on hepatic function of quails. Furthermore, turmeric powder improves immunity in laying hens by increasing total immunoglobulins and especially IgG titers. In addition, the supplementation with curcumin in heat-stressed Hy-Line brown hens increased the activity of antioxidant enzymes and improved immune function, contributing, to the improvement of laying performance and egg quality (Mengjie *et al.,* 2020). Ait-Kaki *et al.* (2021) reported that mixed *Tenebrio molitor* and olive leaves improve quail body weight, reduce feed conversion ratio and did not negatively influence carcass yield and blood parameters of Japanese quail.

The main objective of the present work is to study, for the first time in Algeria, the possibility of using *Tenebrio molitor* insects (yellow mealworms) in *Lohmann Brown* laying hens feed, in order to reduce using soybean as protein feed resources. The second objective is to study the relevance of using curcuma and thus its medicinal properties to improve yellow mealworms digestion.

MATERIALS AND METHODS

The present work was performed in a private poultry farm in the Chemini region (province of Bejaia). Due to the lack of animal ethics commission in Algeria, the authors followed the regulations applied in the Liege University (Belgium).

Animals and Housing

One hundred 36-weeks-old *Lohmann Brown* laying hens were used in this study. They were randomly distributed in four groups of 25 hens each. The first group (Control) received a standard commercial diet (SCD), while the other test groups (TM, TP, and TM-TP) received a SCD diet containing respectively 5% of TM, 0.50% of turmeric powder, and 5% TM and 0.50% of curcuma.

A total of twenty lodges were used to house the 100 hens, i.e., five hens by a lodge whose dimensions are 240 × 80 × 200 cm, provided with 65 cm long perch. Hens were breaded in 16 hours of light, with a continuous nighttime rest period without artificial light (8 hours). The experience has been carried in 2020 for 8 weeks (from January 12 to March 12) and began about 14 days after hens' installation. Rations and water were distributed in "bucket" feeders, *ad-libitum.* Four isoenergetic (3000 kcal per kg dry matter) and isonitrogenous (27.10% of dry matter) experimental diets were formulated for this study. The composition of diets tested here is resumed in Table 1.

Data Collection

Hens were weighed at the beginning and at the end of the experiment. The eggs were collected once a day to determine laying rate. They had been numbered, weighed and the amount of feed consumed was also recorded during all the experimental period to determine feed intake.

Eggs physical characteristics were tested after 4 and 8 weeks of the experiment, on 40 eggs from each group at each week; i.e., 8 from each pen. However, cholesterol and essential fatty acids (docosahexaenoic acid: DHA; and α-linolenic acid: ALA) were dosed once, at the end of the experiment (8 weeks), on 10 eggs from each group; i.e., 2 eggs from each pen.

Eggs Physical Characteristics

Eggs were numbered and weighed with an electronic precision balance of 0.001 g. The maximum force of egg shell rupture (Solidity of the egg shell) was supported using a device: Universal Tensile System (Moula *et al.,* 2009), which exerts pressure on the shell and takes the measurement when the shell begins to crack. The thickness of the shell was determined, using a 0.001 mm precision micrometer, from the average of 3 measurements (taken from the equatorial part). The albumen weight was then determined by subtracting the weight of the yolk and the shell from whole egg weight (Hartmann *et al.,* 2003). The height of the albumen was obtained with a 0.001 mm precision tripod and Haugh units (HU) were calculated (Roberts, 2004):

$$HU = 100 \log (H - 1.7\, x\, W0.37 + 7.6)$$

Cholesterol Content

The whole egg cholesterol content was measured at the end of the experiment, on 10 eggs from each group, using spectrophotometer FoodLab ® Analyzer. Yolks and albumens were mixed; then 5 µl of samples were introduced and mixed in a spectrophotometric cuvette prefilled with a reagent. After 5 minutes of incubation, a second reagent was added and mixed and the measurement was carried 3 minutes later. The enzymes present in the reagents allow a reaction between the cholesterol and a phenol derivative, forming so a pink color complex measured at 505 nm and which is proportional to a cholesterol concentration.

Essential Fatty Acids Analysis

The dosage of two essential fatty acids (DHA and ALA) was realized by the gas chromatography (GC). The yolk of 10 eggs from each group recovered at the end of the experiment was subject of these analysis three times. Before launching essential fatty acids GC analysis, a standard solution containing a methyl ester C15 (Pentacyclic acid or pentadecanoic acid) fatty acid (100 µg/ml) was used to test the accuracy of GC analysis. Egg yolk was first dried by lyophilization. 50 mg egg yolk powder was weighed into 20 ml Pyrex tubes for each sample, supplemented then by 1 ml of a working standard solution. The esterification of fatty acids contained in the egg yolk, was carried by 2 ml of a methanolic

Table 1. Composition of experimental diets

Ingredients (%)	CTRL	TM	TP	TM–TP
Corn	62.5	60	62.5	60
Wheat	3	2	3	2
Lupin seed	4	1	4	1
Bran	4	9	3.5	8.5
Soybean meal	18	15	18	15
Rapseed meal	2	1.75	2	1.75
Limestone	5	5	5	5
Dicalcium phosphate	0.80	0.55	0.8	0.55
Salt	0.25	0.25	0.25	0.25
Choline-(Chloride (50%)	0.08	0.08	0.08	0.08
DL-Methionine	0.1	0.1	0.1	0.1
Mineral Mix[1]	0.11	0.11	0.11	0.11
Vitamin Mix[2]	0.11	0.11	0.11	0.11
Phytase	0.05	0.05	0.05	0.05
Tenebrio molitor	0	5	0	5
Tumeric powder	0	0	0.5	0.5
Chemicial Composition				
Dry matter (%)	87.28	87.41	87.30	87.43
Crude protein (%)	27.04	27.18	27.00	27.14
Ether extract (%)	2.62	3.70	2.63	3.71
Crude fiber(%)	17.30	16.78	17.27	16.74
Crude ash(%)	11.19	10.88	11.17	10.86
P(g/kg)	4.90	4.55	4.89	4.54
ME (Kcal/kg)	2974	3031	2983	3040

[1]Mineral mixture provided following nutrients per kg of diet : Fe, 48 mg; Mn, 72 mg; Cu, 5 mg; I, 1 mg; Se, 0.18 mg; Co, 0.24 mg. [2]Vitamin mixture provided following nutrients per kg of diet : vitamin A, 12,000 IU; vitamin D3, 3,000 IU; vitamin E, 21 IU; vitamin K3, 2.4 mg; vitamin B1, 1.2 mg; vitamin B2, 4.8 mg; vitamin B6, 2.4 mg; vitamin B12, 0.02 mg; niacin, 15 mg; pantothenic acid, 10 mg; folic acid, 0.3 mg; ME: metabolizable energy; CTRL, a standard commercial diet (SCD); TM, 5% of dried TM (*Tenebrio molitor* L. *larvae*); TP, 0.50 % of turmeric powder; TM-TP, 5% of dried TM larvae and 0.50% of turmeric powder.

basic solution (0.5 N) or a sodium methanolate (CH_3ONa). The tubes were closed hermetically, vortexed and then incubated for 20 minutes at 75°C. Once samples were cooled, 1 ml of distilled water was added in order to separate the phases. One milliliter of hexane was added to remove the lipophilic part more easily and then dried by spelling it to tubes containing anhydrous sodium sulfate. One milliliter of this fatty acids extract was recovered, placed in an injection vial, and injected for GC analysis (Harvey, 2019).

Statistical Analysis

SAS software (SAS Institute 2001) was used for all statistical analyses. The statistical analyses were performed to compare the traits in laying hens of different experimental diet groups, using the least squares method of the GLM procedure (SAS, 2001) according to the following linear model:

$$y_{ijk} = \mu + a_i + b_j + (ab)_{ij} + e_{ijk},$$

Where: y_{ijk} trait measured; μ overall mean; a_i effect of diet; b_j effect of week; $(ab)_{ij}$ Effect of groups and age interaction; e_{ijk} random error.

Average eggs laying rate were compared using a χ^2 test. The statistical significance level is set at $P<0.05$.

RESULTS AND DISCUSSION

Hens Weight

Figure 1 reports the hen's bodyweight in the beginning and the end of the experiment. At the beginning of the experiment, the average weights of the hens were around 2080 ± 13.6g (P>0.05). However, at the end of the experiment, the hen's average weights have differently dropped to reach 2008; 2006; 2073; and 2052 g, for CTRL, TM, TP, TM-TP groups, respectively. Only in hens of CTRL and TM, this weights drop was significant (2064 *vs*. 2008 g; and 2097 *vs*. 2006 g; P<0.001, respectively). In contrast, no significant difference was recorded between the beginning and the end of the experiment for the other tested groups, i.e., TP (2095 *vs*. 2073g; P>0.05) and TM-TP (2074 *vs*. 2052g; P>0.05).

The average weight of hens in the CTRL and TM groups decreased significantly (P<0.01) at the end of the experiment compared to the beginning of the experiment (Figure 1). The weights of the hens in the TP and TM-TP groups at the end of the experiment remained similar to those at the beginning of the experiment (Figure 1). The weight stability recorded during the whole experiment period of TP and TM-TP groups, could be due to the anti-inflammatory effect of the turmeric powder supplemented in the feed of these 2 groups. Indeed, turmeric powder is known for its medicinal properties improving the intestinal nutrients absorption and chicken's immunity (Guil-Guerrero *et al.,* 2017; Nawab *et al.,* 2019).

Feed Intake And Egg Laying Rate

The figure 2 presents the feed intake at week 4 and 8 and the whole experiment period. The average feed intake was not significantly affected by diets in the corresponding four groups (123.42 g per a hen; P>0.05), which confirm that supplementation of yellow mealworms and curcuma did not affect palatability. In other side, the stability of hens weight in TP and TM-TP groups while the feed consumption was the same in all tested groups, could confirm the hypothesis of the favorable effect exerted by curcuma on hens' immune and digestive health.

Figure 3 reports the egg-laying rate at week 4 and 8, and the whole experiment period. The average egg-laying rate per group, as reported in Figure 3, ranged from 73.88 to 76.20% with an average of 74.91% for all groups, without significant differences recorded (P>0.05).

Globally, a significant positive effect (P<0.05) of turmeric powder and *Tenebrio molitor* larvae supplementation in hens of TM-TP group was recorded, after four weeks and the whole experimental period, on all eggs physical parameters studied in this work. Furthermore, this same effect was found also in the case of TP group but only on shell thickness and eggs freshness. However, a negative effect was found on eggs weight of hens fed with TM group (Table 2).

The egg weight is influenced by many factors, including the hen age, weight, breed / strain, laying rate, the livestock system, etc. (Moula *et al.,* 2009, 2019; Ledvinka *et al.,* 2012). In addition to these factors, the effect of diet had been confirmed in the present work. In fact, the TM-TP group had produced eggs with an average weight of 68.09 g, significantly (P<0.01) heavier than the eggs of the CTRL (65.45 g), TM (62.88 g) and TP groups (66.52 g). The TM-TP group produced also best eggs quality in term of shell thickness (33.84 cm); rupture force (36.84 N); and freshness (82.36). Moula *et al.* (2009; 2019) reported a positive and significant correlation between these three last sited egg physical parameters.

Egg Cholesterol and Essential Fatty Acids Content

In the present study, eggs recovered from the fours tested groups hens at the end of the experiment contain on average 0.336 mg/100 g of the whole egg (Table 3). The hens in the feed with TM-TP, had given eggs with less than 5.25% cholesterol (P<0.05) than the hens fed SCD (CTRL). Given the controversy over cholesterol in the human diet, this result could present a marketing argument for eggs from hens fed with *Tenebrio molitor* and turmeric powder. Furthermore, the mean of essential fatty acids, DHA and ALA were between 173 and 274 µg/ml; for all groups (Table 3). It is difficult to correlate this similarity (P>0.05) in fatty acids pro-

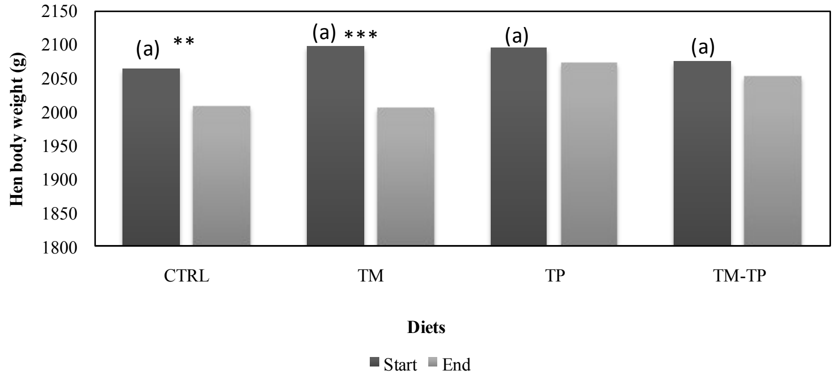

Figure 1. Hens body weight at the beginning and the end of the experiment.
("**" indicates a p-value < 0.01; "***" indicates a P-value <0.001 between the body weight at the beginning and the end of the experiment), CTRL, a standard commercial diet; TM, 5% of dried TM (*Tenebrio molitor* L. larvae); TP, 0.50% of turmeric powder; TM-TP, 5% of dried TM larvae and 0.50% of turmeric powder.

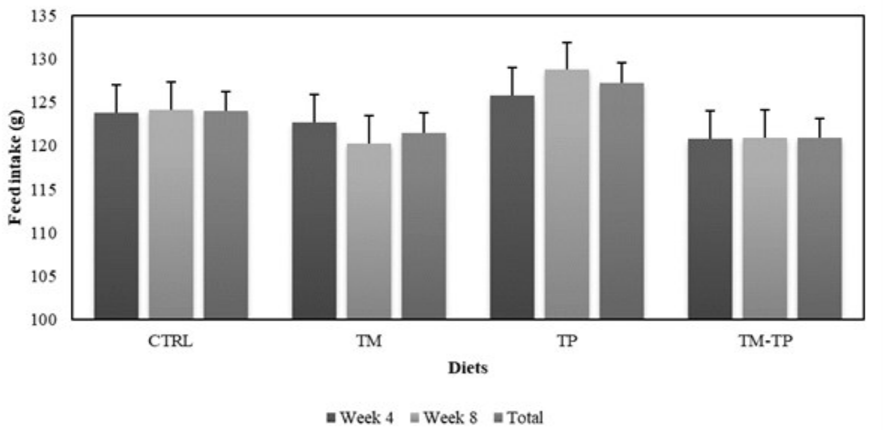

Figure 2. Average daily feed intake (g) of laying hens, after 4 weeks, 8 weeks, and the whole experiment period (CTRL, a standard commercial diet; TM, 5% of dried TM (*Tenebrio molitor* L. *larvae*); TP, 0.50 % of turmeric powder; TM-TP, 5% of dried TM larvae and 0.50% of turmeric powder)

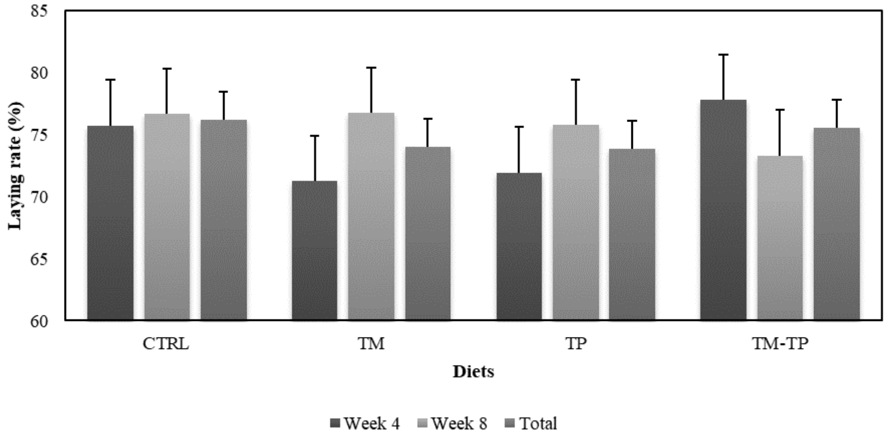

Figure 3. Average eggs laying rate (%), after 4 weeks, 8 weeks, and the whole experiment period (CTRL, a standard commercial diet; TM, 5% of dried TM (*Tenebrio molitor* L. *larvae*); TP, 0.50 % of turmeric powder; TM-TP, 5% of dried TM larvae and 0.50% of turmeric powder)

Table 2. Eggs physical parameters of laying hens receiving commercial diet, *Tenebrio molitor L. larvae* (TM), turmeric powder, or TM larvae and turmeric powder

Traits	Diets				SEM	*P-value*	
	CTRL	TM	TP	TM-TP		Week	Diet
Egg weight (g)	65.45^{a}	62.88^{b}	66.52^{a}	68.09^{c}	0.389	0.015	<0.001
Shell thickness (x10^{-2} cm)	31.52^{a}	32.60^{b}	32.10^{b}	33.84^{c}	0.314	0.002	<0.001
Haugh Units	80.31^{a}	80.84^{ab}	82.23^{b}	82.36^{b}	0.584	<0.001	0.030
Maximal shell breakage strength (N)	34.51^{a}	35.60^{b}	35.10^{a}	36.84^{c}	0.343	0.015	<0.001

^{a,b,c} Means in the same row without common letter are different at P<0.05; CTRL, a standard commercial diet; TM, 5% of dried TM (*Tenebrio molitor* L. *larvae*); TP, 0.50% of turmeric powder; TM-TP, 5% of dried TM larvae and 0.50% of turmeric powder; SEM: Standard error of the mean.

Table 3. The concentration of cholesterol (mg / 100 g); and essential fatty acids (DHA; ALA: µg/ml) in eggs of laying hens receiving standard commercial diet, *Tenebrio molitor L. larvae* (TM), turmeric powder, or TM larvae and turmeric powder

Traits	CTRL	TM	TP	TM-TP	SEM	*P-value*
Cholesterol	0.346^{a}	0.345^{a}	0.331^{ab}	0.321^{b}	0.006	0.048
ALA	175	174	173	174	4.81	0.991
DHA	269	270	271	271	3.13	0.968

^{a,b} Means in the same row without common letter are different at P<0.05; CTRL, a standard commercial diet; TM, 5% of dried TM (*Tenebrio molitor* L. *larvae*); TP, 0.50 % of turmeric powder; TM-TP, 5% of dried TM larvae and 0.50% of turmeric powder; ALA: α-Linolenic acid; DHA: Docosahexaenoic acid; SEM: Standard error of the mean.

file to the four rations because no analysis had been carried for their nutritional composition here. Different results were found in previous studies. Indeed, it had been shown the possibility to get considerable fatty acids amounts such as, multiplying the amount of ALA by 20 (when flax seed oil was added to the ration) or by 6 the amount of DHA, through the addition of fish oil (Shinn *et al.,* 2018; Soliman, 2018).

CONCLUSION

Partial substitution of soybean meal with a mixture of yellow mealworms and curcuma is beneficial for laying hens. It keeps their body-weight stable during production period without affecting laying rates, and the feed ingested amounts. For eggs physical and nutritional quality, the mixture improves significantly their weight, shell thickness and strength, and freshness, while reducing cholesterol content. Yellow mealworms and curcuma could be a beneficial alternative to soybean in laying hens' diet. Eggs producers in Algeria should adopt this alternative to reduce their dependence to imports and the international market, and improve the physical and the nutritional quality of eggs.

CONFLICT OF INTEREST

The author declares no conflict of interests.

ACKNOWLEDGMENT

The authors thank Moula Hachemi, Moula Samir, and Moula Mokrane for their help during the conduct of this study in the private farm. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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