Combined use of *Candida Utilis* and *Idesia polycarpa* var. *Vestita* Fruit Improve the Production Performance of Laying Quail

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Abbreviated Title: A new feeding raw material applying on laying quails.

ABSTRACT: *Idesia polycarpa* Maxim. var. *vestita* Diels (*Idesia polycarpa*), which is widely distributed in south China, is still underexplored. This study applied *Idesia polycarpa* defatted fruit (IPF) and *Candida utilis* to the feed of laying quails using solid-state fermentation. In comparison to the standard diet group, birds fed with mixture of IPF and *candida utilis* (MIC) showed better production capacity, and the ML group (1% MIC added) achieved the greatest egg mass (9.77 on average; $P<0.01$) and laying rate (87.7% on average; $P<0.01$). Compared to the standard diet group, the cholesterol content was lower in both the ML (1% MIC addition groups) and IL (1% IPF addition groups), and 5% MIC added group had higher n-3 polyunsaturated fatty acid content. Furthermore, birds given the MIC dietary supplement showed a thicker jejunum wall than the standard diet group. In addition, the related mRNA expression of SRBEP-1, SREBP-2, ATGL, APOVLDL-II which are involved in the fatty acid and cholesterol biosynthesis suggested that the addition of *candida utilis* could effectively improve the production capacity of laying quails while decrease the negative effects of IPF. This work also demonstrates how MIC can be applied to improve the production of laying quails.

KEYWORDS: *Candida utilis, Idesia polycarpa fruit, Laying quails, Egg production,.*

Introduction

Idesia polycarpa (*Idesia polycarpa* Maxim. var. *vestita* Diels) is widely distributed in south China, and it is known for its high-quality edible oil, which has a high content of conjugated linoleic acid (CLA). Moreover, the application of *Idesia polycarpa* oil in biodiesel production has been demonstrated (1, 2). There have been several studies on *Idesia polycarpa* fruit, including functional research on *Idesia polycarpa* fruit extraction and studies on the improvement of the extraction process. The remarkable antioxidant qualities, anti-skin-aging ability (3) and anti-adipogenic ability (4) of *Idesia polycarpa* extracts have been demonstrated, and an optimized extraction method has also been established (5). However, reports on the application of *Idesia polycarpa* fruit for the other purposes are still limited. In this study, we are tried to apply *Idesia polycarpa* defatted fruit (IPF) in laying quail's breeding, and for purpose of effective use, solid-fermentation was selected.

From a historical perspective, phytogenic materials fermented with specific microbe are recognized to benefit farm animals and are described as far back as the beginning of the last century (6, 7). Even now, fermentation is still gaining interest because of its important role in the production of quality feed and the utilization of unexploited feed materials (8, 9). Moreover, based on existing studies, the addition of probiotic could effectively decrease anti-nutritive factors and allergenic proteins (10, 11). Furthermore, fermented feed represents a plausible alternative to traditional antibiotics, as it enhances a number of important processes in animals (12, 13).

Candida utilis is an important member of edible yeast, which has safely been

used as a food or feed additive in food and feed industry for years. *Candida utilis* is able to robust growth on inexpensive nitrogen and carbon sources, such as pentoses (14). The main product of *Candida utilis* is single-cell protein, which has excellent nutritional properties and has an agreeable odor and flavor, and this odor and flavor can also be conferred to product by fermentation (15, 16). Therefore, *Candida utilis* is a good choice in our study.

As an important product in the poultry industry, quail eggs are gaining increasing interest thanks to their good taste, health benefits and nutrient richness(17, 18). In addition, as a sensitive experimental animal (19), *Coturnix japonica* and its eggs are often used in research (20).The effects of various kinds of materials have been tested on the production of quail eggs, for instance, soybean (21), cassava meal (22), and sunflower meal (23). However, no reports are available regarding the effects of *Idesia polycarpa* products on the quality of quail eggs.

In this study, mixture of IPF and *candida utilis* (MIC) was added to the feed of laying quails as a probiotc additive. Production capacity, health performance and egg quality of laying quails were analysed during or after the feeding phase. Moreover, the relative mRNA expression level of SRBEP-1, SREBP-2, ATGL, APOVLDL-II which are involved in the fatty acid and cholesterol biosynthesis are measured.

Materials and methods

Probiotic and Feed

Candida utilis was purchased from China Center of Industrial Culture Collection

(31170, CICC).

Idesia polycarpa defatted fruits (IPF) which obtained by physical cold pressing process were kindly supplied by Agriculture Bureau of Jianyang, China, and processed into a dried powder before it be used.

Mixture of *Idesia polycarpa* defatted fruits and *Candida utilis* (MIC) was produced by a solid fermentation method (solid-liquid ration 1:1.2; ammonium nitrate 8.5%; inoculum size 10.5% (*Candida utilis* in suspension form, 1.0×10^7 colony forming units (cfu)/ml); temperature 30℃; time 4d;), and processed into a dried powder before it be used.

Basal feed (BD, Tequ302, Sichuan) in powder form which contains all nutrients necessary for animals was purchased in the local market. Nutrients of MIC, IPF and basal diet are shown in **Table 1**.

Birds and Experimental Design

All animal protocols were approved by the Sichuan University animal Care and Use committee. A total of 250 layer quails (*Coturnix coturnix japonica*, 90 days old) from a local breeding farm (Chengdu, China) were randomly divided into 5 groups: Control, base diet only; MH, basal diet supplemented with 5% MIC; ML, basal diet supplemented with 1% MIC; IH, basal diet supplemented with 5% IPF; IL, basal diet supplemented with 1% IPF. All birds were kept in a room with a relatively stable temperature (24-30 ℃) and humidity (65%). During the 8-week experimental period, birds had access to water *ad libitum*, but with a fixed amount of diet (1,000 g/d, equivalent to a dose of 20 g/d/bird), which based on the local farm feeding management for this season. In addition, from week 0 to 2, a trace amount of MIC (0.5g (0.05%) per day) was added into the daily diet of each group to help quails

adaptation to the peculiar smell of the feed，except the control group. The quail's differentiated diet was supplied during the 6 weeks feeding test period from week 3 to week 8. The feeding experimental design is shown in **Table 2**.

Sample collection

The number of eggs was counted every day, and the results are represented as the mean value of each week. Eggs were randomly collected (five eggs from each group, 30 eggs in total) each week to measure the egg quality during the experimental period. The egg weight, density, and axis ratio, as well as the egg yolk weight and the ratio between the egg weight and egg yolk weight were measured. The Haugh unit values were calculated using the Haugh unit formula based on egg weight and albumen height, as determined using a Vernier caliper. The eggshell thickness and membrane thickness were determined as the mean value of measurements taken at three locations on the egg (sharp end, blunt end, and middle section).

The egg yolk samples were separated from the rest of the egg for triglyceride and cholesterol determination on weeks 6 to 8. The yolk triglycerides and cholesterol were determined using ELISA (SMP500-15859-SBRE, Molecular Devices, USA) using commercial kits according to the manufacturer's protocol (Jiancheng, Nanjing, China). On week 8, three additional eggs/each group were collected for yolk lipid extraction.

At the end of the Phase 2 (week 8), three quails in each group were randomly sacrificed by cervical dislocation for sample collection after weighing. Tissue samples, including those from the intestine, interclavicular fat pad and liver, were immediately

106 collected from all 15 quails after they were sacrificed. The same segment of the
107 intestine was collected and stored in a prepared fixative (10% formalin) for histology
108 analysis, the fat pads were immediately weighed, and both the fat pad and liver
109 samples were snap-frozen in liquid nitrogen and stored at -80 ℃ until the subsequent
110 RNA isolation.

111 **Yolk lipid extraction**

112 Yolk samples were separated from boiled eggs. Yolk lipids were extracted from
113 three yolks in each group using hexane extraction (AOAC 2003. 06). The extracted
114 yolk lipids were stored in -20 ℃ until the subsequent fatty acids analysis.

115 **Methylation and GC/MS**

116 The methylation of the yolk lipids was determined using the alkali catalysis
117 method (24). The peak area and fatty acid percentages were calculated using the
118 GCMS-solution software (Shimadzu, Japan), and the fatty acid methyl esters are
119 expressed as the percentage of the total fatty acids.

120 **Jejunum morphology**

121 After being fixed in 10% formalin for 24 h, samples of the jejunum tissues were
122 selected for 5 μm-thick paraffin sections and stained with hematoxylin-eosin (HE).
123 The analysis included well thickness, villus height and crypt depth and was performed
124 using a microscope equipped with a camera and connected to a computer with the
125 appropriate software (Olympus, BX53, Cell^B software, Japan). Data were collected

126 from at least five randomly selected visible regions under the microscope. Mean
127 values of three birds per group were calculated for the subsequent statistical analysis.

128 **RNA isolation**

129 The total RNA was extracted from the fat pad and liver tissue using TRIzol
130 reagent (TakaRa, USA). The RNA quality and quantity were determined using a
131 Nanodrop 2000 (Thermo Scientific, USA). The 260/280 absorption ratios of all
132 extracted samples were between 1.8 and 2.0, and the RNA integrity was further
133 analyzed using gel electrophoresis. The first-strand cDNA was immediately
134 synthesized using the PrimeScript RT reagent kit (TakaRa, USA) following the
135 manufacturer's instructions. The obtained cDNA was stored at -20 ℃ until the
136 subsequent real-time PCR analysis.

137 **qRT-PCR analysis**

138 The primers used for target gene amplification were either designed using primer
139 3.0 based on quail-related gene sequences or reported by specific references (**Table 3**).
140 β-actin was selected as the housekeeping gene to normalize the target gene expression.
141 All primers in this study were synthesized by Tsingke Biotech (Chengdu, China). The
142 specificity of each of the designed primers was checked via gel electrophoresis
143 analysis and melting curve analysis during quantitative real-time PCR.
144 The relative quantification of all transcripts was performed using qRT-PCR with
145 the Bio-Rad CFX96 Real-Time PCR System (Bio-Rad Laboratories, USA). Real-time

quantitative PCR was performed with SsoFast EvaGreen Supermix (Bio-Rad Laboratories, USA). A total volume of 10 µl containing 5 µl of SsoFast EvaGreen Supermix, 3 µl of RNA-free water, 0.5 µl of forward primer, 0.5 µl of reverse primer, and 1 µl of template cDNA was used. PCR amplification was carried out as follows: denaturation at 95 ℃ for 30 s, followed by 40 cycles at 95 ℃ for 5 s and a specific annealing temperature of 55 ℃ for 30 s. The $2^{-\Delta\Delta CT}$ method was used to evaluate the mRNA expression levels (25).

Statistical analysis

The statistical analysis was conducted using the SPSS software (SPSS, IBM, version 20). The standard error of the means (SEM) was obtained from the analysis of variance (ANOVA) that was conducted. The significance of the differences among means was determined using one-way ANOVA and compared using Dunnett's multiple range tests. The results were considered statistically significant at $P>0.05$.

Results

Jejunum morphology

In this study, all birds purchased from the local farm at the age of 90 days were no different from the normal farmed birds. The jejunum morphology analysis was performed in week 8 (**Table 4; Fig. 1-I**). Compared to control, the intestinal wall thickness was significantly increased in the MIC groups; it was approximately 79 µm and thus extremely thicker than that in the other groups ($P<0.01$). Meanwhile, The

166 villus height showed a significant increase both in MIC and IPF groups. Group MH
167 and ML showed a clear enhancement of the intestinal wall, villus height and crypt
168 depth. In addition, the intestinal wall thickness was 43 μm in the IPF groups, similar
169 to the control group (41 μm), and in contrast to the increased villus height, the crypt
170 depth was decreased.

171 **Yolk cholesterol and fatty acids**

172 The results of the yolk cholesterol and triglycerides determination are shown in
173 **Table 5**. In this study, compared to the control, the groups with lower amount of
174 additive showed a decrease in the yolk cholesterol content, but there were no effects
175 on the triglycerides. A decrease in yolk cholesterol was observed in groups ML and IL
176 compared to the control (P<0.01), while the 5% groups (MH and IH) showed no
177 significant change. In addition, the most significant yolk cholesterol decline was
178 observed in group ML.

179 In our study, both the MIC and IPF contained few lipids (**Table 1**). The
180 conjugated linoleic acid content was in agreement with the results of Yang et al. (2):
181 68.64% in IPF oil and 72.21% in MIC oil (**Table 6**). The composition of the yolk fatty
182 acids is shown in **Table 7.**

183 As shown in **Table 7,** the n6/n3 (n3-polyunsaturated fatty acids/
184 n6-polyunsaturated fatty acids) ratio was significantly higher in IPF groups than in the
185 control (P<0.01). Conversely, compared to the control, the decrease in the n6/n3 ratio
186 in group MH was significant (P<0.01), and group MH also showed an increased n3

187 (EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid) content and decreased n6
188 content, which are beneficial to the absorption of unsaturated fatty acid (26).

189 **Egg production and quality**

190 The effects of MIC and IPF dietary supplements on quail eggs were measured
191 (**Table 8**). The yolk weight, shell thickness, Haugh unit and diameter ratio were
192 similar among the groups. The yolk weight was approximately 0.29-0.33% of the total
193 weight of the egg, the shell thickness was 0.16-0.18 mm, the Haugh unit was 79-84,
194 and the diameter ratio was 0.77-0.82.

195 Egg production in the MIC groups generally increased during the whole
196 experiment period compared to the control (**Table 9**). Group ML achieved a
197 significant increase in egg mass and laying ratelaying rate on average, higher than
198 control group during the same period of time. The average egg mass in group ML was
199 9.77 g/d/bird, which was significantly higher than that in the other groups. In contrast,
200 the egg mass decreased in the IPF groups; group IH (5% IPF added) exhibited a
201 significant production decrease that was more clear in the last week compared to the
202 control (6.06 g/d/bird; $P<0.01$). Meanwhile, laying ratelaying rate in the MIC groups
203 generally increased during the whole experiment period compared to the control, and
204 decreased laying ratelaying rate was observed in the IPF groups (**Table 9**). Similarly,
205 group ML achieved a significant increase in laying ratelaying rate, at 87.7% on
206 average, which is approximately 12% above the control. In group ML, nearly 44 eggs
207 per day were produced on average, while the control group produced 38 eggs per day

during the same period of time.

Genes

The relative mRNA expression level changes in SRBEP-1, SREBP-2, ATGL, APOVLDL-II, which are involved in fatty acid and cholesterol biosynthesis were measured. The relative mRNA expression of group ML and IL were showed in **Fig. 1(**II, III, IV**)**. Group ML and group IL are showed similar relative mRNA expression changes. Compared to the control, both ML and IL groups showed significant up-regulation of APOVLDL-II in liver (**Fig. 1-III**, $P<0.01$). SREBP-1 was up-regulated in the fat pad ($P<0.01$) but did not significantly change in the liver, while SREBP-2 was significantly down-regulated in the fat pad and up-regulated in the liver. However, the up-regulation of ATGL was found in ML.

Discussion

In the poultry industry, the wide use of antibiotics usually results in a thinner intestinal wall, smaller total villus area, and shorter villus height and crypt depth (27). In this study, all birds purchased from the local farm at the age of 90 days were no different from the normal farmed birds. The intestinal wall thickness was significantly increased in the MIC groups; it was approximately 79 μm and thus extremely thicker than that in the other groups ($P<0.01$). The villus height of both MIC and IPF groups showed a significant increase, and this increase may related to the activation of intestinal villi growth (28). MIC groups showed a clear enhancement of the intestinal

228 wall, villus height, suggesting that the birds of MIC groups have better digestive and
229 absorptive capacity, and the appropriate amount of MIC additive was beneficial for
230 the intestinal health of laying quails. However, IPF showed no effect on the intestinal
231 wall, the intestinal wall thickness was 43 μm in the IPF groups, similar to the control
232 group (41 μm).

233 MIC and IPF supplementation also changed the cholesterol content of egg.
234 Cholesterol is important for many physiological functions (29-31), and as a
235 triglyceride, cholesterol is also an essential material for yolk formation and egg
236 production. However, high cholesterol intake must be avoided in our diet because
237 excess cholesterol is related to cardiovascular disease and nonalcoholic steatohepatitis
238 which contributing to hepatocellular carcinoma, thus increasing the risk to human
239 health (32-34). Moreover, cholesterol content is used in the evaluation of egg quality
240 in the poultry industry. From the perspective of human health, eggs with lower
241 cholesterol are considered to have superior quality, and more attention is currently
242 paid to reducing egg cholesterol (35, 36). In this study, compared to the control, the
243 groups with lower amount of additive showed a decrease in the yolk cholesterol
244 content, but there were no effects on the triglycerides. The most significant yolk
245 cholesterol decline was observed in group ML. These results indicate that both MIC
246 and IPF were effective in terms of affecting the yolk cholesterol, and the optimal
247 addition is1%.

248 Ouyang et al. found that a different oil supplement given to hens was able to
249 change the cholesterol and polyunsaturated fatty acid content in the yolk (37).

Furthermore, in a study conducted by Aydin et al., dietary conjugated linoleic acid (CLA) added to hen diets significantly changed the fatty acid composition of the yolk (38). In our study, both the MIC and IPF contained few lipids (**Table 1**). The conjugated linoleic acid content was in agreement with the results of Yang et al (2), and this small amount of CLA might be involved in the change of bird partly (**Table 6**).

n-3 polyunsaturated fatty acids and n-6 polyunsaturated fatty acids is indispensable fatty acid for human(39). It is well known that a lower n6/n3 ratio is better according to the guidelines of the WHO regarding healthy food for humans (26). As shown in **Table 7**, compared to the control, the decrease in the n6/n3 ratio in group A was significant (P<0.01), and group MH also showed an increased n3 (EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid) content and decreased n6 content, which are beneficial to the absorption of unsaturated fatty acid (26). Conversely, the n6/n3 ratio was significantly higher in IPF groups than in the control (P<0.01).

As we know that both fatty acid and cholesterol metabolism are involved in the regulation of egg formation (40, 41), and lipid mobilization from the adipose tissue is one of the main sources supporting the onset of egg production. As a result of lipid mobilization, the increase in the fat pad is the most intuitive indication of a supply of raw material available for egg production (42). The relative mRNA expression level changes in SRBEP-1, SREBP-2, ATGL, APOVLDL-II, which are involved in fatty acid and cholesterol biosynthesis (43-45), were measured at the end of the study.

Adipose triglyceride lipase (ATGL) is highly expressed in adipose tissue and

catalyzes the initial step of triglyceride hydrolysis (46). In a study conducted by Chen et al., G0/G1 switch gene 2 (G0S2) overexpression carried out the inhibition of ATGL expression, which resulted in a delayed laying onset and reduced egg production (40). Sterol regulatory element-binding protein-1 and -2 (SREBP-1 and SREBP-2) are SREBPs encoding genes that mainly regulate the homeostasis of lipids and cholesterol, respectively (43, 47). SREBP-1 up-regulation preferentially enhances fatty acid and triglyceride synthesis, while increased SREBP-2 expression promotes cholesterol synthesis (48-50). Apo very low density lipoprotein II (APOVLDL-II) is related to the secretion of VLDL, which plays a pivotal role in egg formation (19). The described changes in the fat pad and ATGL expression found in the study by Chen et al (40) were also observed in our study (**Fig. 1**-III).

In this study, the interclavicular fat pat weight significantly decreased in both the MIC-added and IPF-added groups. In group ML, for example, while the fat pad decreased, the ATGL was up-regulated, that is, the lipid supply for egg formation was enhanced. Meanwhile, as the results show in **Fig. 1(**III, IV**)**, we found that fatty acid synthesis was strengthened in the fat pad with the up-regulation of SREBP-1 and that the synthesis and transport of cholesterol was enhanced in the liver with the up-regulation of SREBP-2 and APOVLDL-II. In addition, the described jejunum morphology results indicate that the birds in group ML had better intestine health, which allowed better digestion and absorption. These changes indicate that the birds in group ML have enhanced energy requirement and material supplementation for egg production during the feeding period (**Fig. 2**). Hence, in the last week, MIC promoted

294 the egg quantity, but the single egg weight was lighter due to the fixed diet, just as the
295 results showed in group ML. These results indicate that while the raw-material
296 supplement supplied for the production of eggs was enhanced in the MIC groups, with
297 an improvement in product performance, the fixed diet cannot meet the requirements
298 of gradually increasing performance.

299 Moreover, the same tendency in gene expression was found in both groups ML
300 and IL, combined with the change in the fat pad, we assumed that IPF facilitated
301 several processes involved in egg production, although it ultimately resulted in some
302 damage to body health. However, based on the results, we believe that *candida utilis*
303 could help to reduce the disadvantage of IPF which was harmful to the laying quails
304 and that MIC, a product of fermentation, was beneficial to intestinal health and could
305 enhance the production capacity of laying quails. Meanwhile, the groups with a lower
306 MIC amount (1%) showed that these amounts were more effective in promoting
307 laying ratelaying rate and egg mass. Furthermore, the results also suggest that through
308 solid-state fermentation, IPF, which is not suitable for direct feeding, could be a good
309 feed additive.

310 Overall, our study showed that MIC improved egg production and quality in
311 laying quails. This study is showing the application of *Idesia polycarpa* fruit and
312 *candida utilis* in feeding. MIC at 1% is the most efficient dosage not only in terms of
313 an increase in egg production but also a decrease in yolk cholesterol. Meanwhile,
314 adding 5% MIC could result in a higher EPA and DHA content and a better n3/n6
315 ratio. Furthermore, MIC was beneficial to the health of the quail's intestine and

316 reduced the potential negative effects of industrial feeding.

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Notes

The authors declare no competing financial interest.

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Tables

Table 1. The Main Nutrients of MIC, IPF and Basal diet (%)

	MIC	IPF	Basal diet
Crude protein	16.4	8.49	21
Crude lipid	6.34	13.34	2.5
Moisture content	5.04	7.20	14
Ash	4.62	4.49	12
Free Amino acid	6.81	5.01	
Lysine	0.13	0.09	
Methionine	0.11	0.05	0.46
Phenylalanine	0.36	0.27	
Threonine	0.31	0.23	
Leucine	0.57	0.44	
Isoleucine	0.34	0.26	
Arginine	0.35	0.15	
Histidine	0.30	0.23	
Valine	0.42	0.33	

MIC: means Mixture of *Idesia polycarpa* defatted fruit and candida utilis**; IPF**: means *Idesia polycarpa* defatted fruit.

Table 2. Daily Diet Arrangement of MIC, IPF and Control Groups (g)

	Week 0 to 2			Week 3 to 8		
	Basal diet	MIC	IPF	Basal diet	MIC	IPF
MH	1000	0.5	-	950	50	-
ML	1000	0.5	-	990	10	-
IH	1000	0.5	-	950	-	50
IL	1000	0.5	-	990	-	10
Control	1000	0.5	-	1000	-	-

[1]**MIC**: means Mixture of *Idesia polycarpa* defatted fruit and candida utilis**; IPF**: means *Idesia polycarpa* defatted fruit.

[2]**MH**: Basal diet supplemented with 5% MIC, **ML**: basal diet supplemented with 1% MIC, **IH**: basal diet supplemented with 5% IPF, **IL**: basal diet supplemented with 1% IPF, **Control**: Control group, base diet only.

[4]Superscripts **a, b, c, d, e**: Means within the same superscripts without the same row are significantly different ($P<0.05$).

Table 3. Primer Sequences for the mRNA Expression Analysis of Genes

Genes	primer sequence (5'-3')	product size (bp)	Gene Bank
APOVLDL-I	F: CAGTTCTTGCTGGATGTTTCCCAGAC	430	S82591.1
I	R: CAATGGCCAAGTCATTCAGGAGGA		
SREBP-1	F: CTACCGCTCATCCATCAACG	181	NC_029529.1
	R: CTGCTTCAGCTTCTGGTTGC		
SREBF-2	F: CCCAGAACAGCAAGCAAGG	108	XM_416222
	R: GCGAGGACAGGAAAGAGAGTG		
ATGL	F: CAGCAGGACGGTTGGGTATTTC	154	GQ221783.1
	R: CCACGCAAGGTTGGAGGTATCA		
β-actin	F: TGATGGTTGGTATGGGTCAGAAAG	92	NC_029529
	R: ATGTTCAATGGGGTATTTCAAGGT		

Table 4. Jejunum Morphology Analysis of Laying Quails Fed a Basal Diet, MIC Added Diet or IPF Added Diet

	MIC		IPF				
	MH (5%)	ML (1%)	IH (5%)	IL (1%)	Control	SEM	*P*
Intestine wall thickness(μm)	78.5^{a}	79.2^{a}	43.7^{b}	43.4^{b}	41.3^{b}	6.09	<0.01
villous height (μm)	835^{b}	908^{a}	818^{bc}	801^{c}	610^{d}	26.9	<0.01
Crypt depth (μm)	112.8^{b}	189.5^{a}	72.9^{d}	77.0^{d}	91.5^{c}	4.35	<0.01
V/C	7.40^{b}	4.79^{d}	11.22^{a}	10.41^{a}	6.67b^{c}	0.50	<0.01

[1]N=9.

[2]**MIC**: means Mixture of *Idesia polycarpa* defatted fruit and candida utilis**; IPF**: means *Idesia polycarpa* defatted fruit.

[3]. **V/C** means the ratio of villous length/crypt depth.

[4]**MH**: Basal diet supplemented with 5% MIC, **ML**: basal diet supplemented with 1% MIC, **IH**: basal diet supplemented with 5% IPF, **IL**: basal diet supplemented with 1% IPF, **Control**: Control group, base diet only.

[4]Superscripts **a, b, c, d, e**: Means within the same superscripts without the same row are significantly different (P<0.05).

Table 5. Yolk Cholesterol and Triglyceride Content of Laying Quails Fed a Basal Diet, MIC Added Diet or IPF Added Diet

	MIC		IPF				
	MH (5%)	ML (1%)	IH (5%)	IL (1%)	Control	SEM	*P*-value
Yolk Weight (g)	3.61	3.10	3.60	3.29	3.39	0.39	0.73
Cholesterol (mg/g yolk)	13.1^{ab}	12.1^{b}	14.0^{a}	11.2^{b}	14.5^{a}	0.25	<0.01
(mg/ yolk)	46.54^{ab}	37.0^{c}	48.5^{ab}	40.3^{b}	49.1^{a}	4.73	<0.01
Triglyceride (mg/g yolk)	183	175	197	183	190	8.57	0.10
(mg/ yolk)	660.2^{a}	536.0^{b}	682.9^{a}	661.4^{b}	644.4^{a}	69.2	0.01

[1]N=9.

[2]**MIC**: means Mixture of *Idesia polycarpa* defatted fruit and candida utilis**; IPF**: means *Idesia polycarpa* defatted fruit.

[3]**MH**: Basal diet supplemented with 5% MIC, **ML**: basal diet supplemented with 1% MIC, **IH**: basal diet supplemented with 5% IPF, **IL**: basal diet supplemented with 1% IPF, **Control**: Control group, base diet only.

[4]Superscripts **a, b, c, d, e**: Means within the same superscripts without the same row are significantly different (P<0.05).

Table 6. Fatty Acid Composition of MIC Oil and IPF Oil (%)

Fatty Acid		MIC	IPF
Palmitic	C16:0	15.51	17.36
Stearic	C18:0	1.79	1.66
Oleic	C18:1	3.5	4.54
Palmitoleic	C16:1	6.41	7.55
Linoleic	C18:2	72.21	68.64

[1]**MIC**: means Mixture of *Idesia polycarpa* defatted fruit and candida utilis**; IPF**: means *Idesia polycarpa* defatted fruit.

Table 7. Fatty Acid Composition of Yolk Oil extracted from each groups

Fatty acid		Control	MH	ML	IH	IL
Myristic	C14:0	0.45^{b}	0.57^{a}	0.54^{a}	0.43^{b}	0.39^{b}
Palmitic	C16:0	29.53	30.55	31.88	30.92	32.72
Palmitoleic	C16:1;n-7	2.16^{c}	3.64^{a}	2.39^{bc}	2.82^{b}	2.20^{bc}
Stearic	C18:0	12.8^{a}	11.6^{b}	10.5^{c}	9.23^{d}	10.6^{c}
Oleic	C18:1;n-9	33.8^{b}	35.3^{b}	34.6^{b}	37.2^{a}	32.2^{c}
Linoleic	C18:2;n-6	17.1^{b}	14.1^{c}	16.7^{b}	16.8^{b}	18.2^{a}
α-Linolenic	C18:3;n-3	0.22^{a}	0.21^{a}	0.25^{a}	0.17^{b}	0.22^{a}
Arachidonic	C20:4;n-6	2.56^{a}	2.38^{a}	1.88^{b}	1.65^{b}	1.64^{b}
Eicosapentaenoic	C20:5;n-3	0.27^{b}	0.37^{a}	0.29^{ab}	0.14^{c}	0.25^{b}
Docosahexaenoic	C22:6;n-3	0.89^{b}	1.02^{a}	0.76^{c}	0.51^{d}	0.48^{d}
SFA		42.8^{a}	42.7^{a}	42.9^{a}	40.6^{b}	43.7^{a}
MUFA		36.0^{c}	38.9^{b}	37.0^{bc}	40.0^{a}	34.4^{d}
PUFA		21.0^{a}	18.1^{c}	19.8^{b}	19.3^{b}	20.8^{ab}
n-3PUFA		1.38^{b}	1.60^{a}	1.30^{b}	0.82^{c}	0.95^{bc}
n-6PUFA		19.6^{a}	16.5^{c}	18.5^{b}	18.4^{b}	19.9^{a}
n6/n3		14.2^{c}	10.3^{d}	14.3^{c}	22.5^{a}	20.9^{b}

[1]N=3.

[2]**MIC**: means Mixture of *Idesia polycarpa* defatted fruit and candida utilis**; IPF**: means *Idesia polycarpa* defatted fruit.

[3]**MH**: Basal diet supplemented with 5% MIC, **ML**: basal diet supplemented with 1% MIC, **IH**: basal diet supplemented with 5% IPF, **IL**: basal diet supplemented with 1% IPF, **Control**: Control group (base diet);

[4]Superscripts **a, b, c, d, e** Means within the same superscripts without the same row are significantly different ($P<0.05$).

[5]**SFA**: short chain fatty acid; **MUFA**: monounsaturated fatty acid; **PUFA**: polyunsaturated fatty acids.

[6]All results in percentage terms.

Table 8. Egg Quality of Laying Quails Fed a Basal Diet, MIC Added Diet or IPF Added Diet

	MIC		IPF				
	MH (5%)	ML (1%)	IH (5%)	IL (1%)	Control	SEM	P-VALUE
Yolk Weight (%)							
initial	0.30	0.30	0.31	0.30	0.31	0.187	0.980
last week	0.31	0.30	0.33	0.31	0.29	0.029	0.935
average	0.31	0.30	0.32	0.31	0.30	0.119	0.754
Shell Thickness(mm)							
Initial	0.16	0.16	0.16	0.17	0.16	0.007	0.785
Last week	0.17	0.18	0.19	0.18	0.18	0.008	0.285
Average	0.18	0.18	0.18	0.18	0.17	0.008	0.563
Haugh Unit							
Initial	80.59	79.84	80.75	79.59	79.15	2.760	0.827
Last week	79.25	81.18	79.86	80.42	80.78	1.881	0.924
Average	82.92	81.51	84.75	81.26	80.81	1.437	0.458
Diameter Ratio							
Initial	0.77	0.80	0.82	0.79	0.80	0.346	0.805
Last week	0.77	0.78	0.78	0.78	0.79	0.082	0.285
Average	0.78	0.77	0.79	0.78	0.77	0.007	0.269

[1]N=9.

[2]**MIC**: means Mixture of *Idesia polycarpa* defatted fruit and candida utilis**; IPF**: means *Idesia polycarpa* defatted fruit.

[3]**MH**, basal diet supplemented with 5% MIC; **ML**, basal diet supplemented with 1% MIC; **IH**, basal diet supplemented with 5% IPF; **IL**, basal diet supplemented with 1% IPF; **Control**, Control group (base diet).

[4]**Initial**: Mean of week 0 to 2; **Last week**: Mean of week 8; **Average**: Mean of week 3 to 8.

Table 9. Egg Mass and Egg Production of Laying Quails Fed a Basal Diet, MIC Added Diet or IPF Added Diet

	MIC		IPF				
	MH (5%)	ML (1%)	IH (5%)	IL (1%)	Control	SEM	*P*-value
Egg mass (g/d/bird)							
Initial	9.44[a]	9.41[a]	9.31[a]	9.14[a]	9.18[a]	0.30	0.88
Last week	9.18[a]	9.31[a]	6.04[c]	7.82[b]	9.27[ab]	0.30	<0.01
Average	9.41[a]	9.77[a]	6.06[d]	7.86[c]	8.87[b]	0.38	<0.01
Laying rate (%)							
Initial	81.0[a]	81.3[a]	80.7[a]	80.7[a]	79.3[a]	2.57	0.97
Last week	80.1[b]	90.2[a]	56.3[e]	68.1[d]	78.4[c]	2.91	<0.01
Average	82.2[a]	87.7[a]	62.4[c]	72.3[b]	76.0[b]	3.15	<0.01

[1]N=50.

[2]**MIC**: means Mixture of *Idesia polycarpa* defatted fruit and candida utilis**; IPF**: means *Idesia polycarpa* defatted fruit.

[3] **MH**, basal diet supplemented with 5% MIC; **ML**, basal diet supplemented with 1% MIC; **IH**, basal diet supplemented with 5% IPF; **IL**, basal diet supplemented with 1% IPF; **Control**, Control group (base diet).

[4]Superscripts **a, b, c, d, e** Means within the same superscripts without the same row are significantly different (P<0.05).

[5]**Initial**: Mean of week 0 to 2; **Last week**: Mean of week 8; **Average**: Mean of week 3 to 8;

Figures

Fig. 1

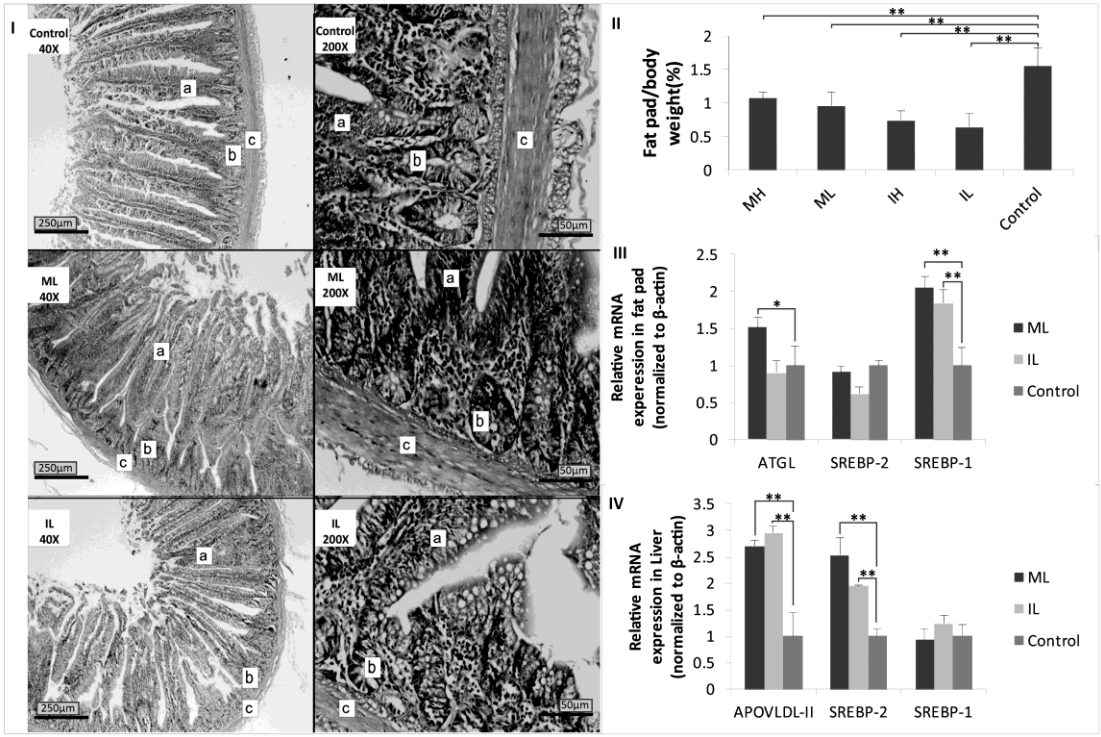

Fig. 1 Results of jejunum morphology, fat pad, and relative mRNA expression.

I: Tissue sections of jejunum. **ML**: the Tissue section figure of group ML; **IL**: the Tissue section figure of group IL; collected in 40×amplifications and 200×amplifications respectively.

II: Ratio of Fat Pad to Body Weight（%）. **MH**, basal diet supplemented with 5% MIC; **ML**, basal diet supplemented with 1% MIC; **IH**, basal diet supplemented with 5% IPF; **IL**, basal diet supplemented with 1% IPF; **Control**, Control group (base diet). **Means significant relative to control (P<0.01).

III: Relative mRNA expression of 1% MIC added group (**ML**) and 1%IPF added group (**IL**) in fat pad. ****Means significant relative to control (P<0.01); ***Means significant relative to Control (P<0.05).

IV: Relative mRNA expression of 1% MIC added group (**ML**) and 1%IPF added group (**IL**) in liver. ****Means significant relative to control (P<0.01).

Fig. 2

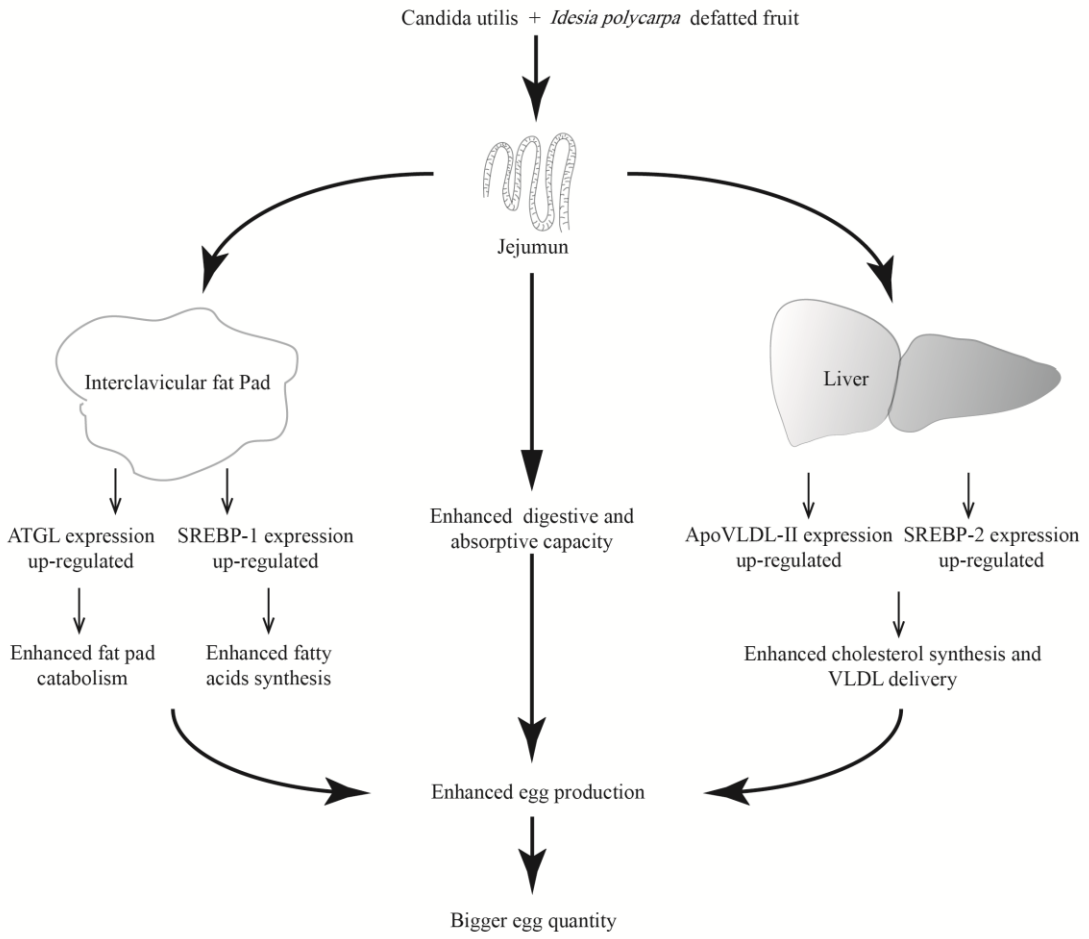

Fig. 2 The influence of MIC in laying quails. Compared to the control, the intestine statues of laying quails was significantly changed with the addition of MIC, as well as the relative mRNA expression in the fat pad and liver, ultimately, these changes result in an enhanced egg production.