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The Effect of Dietary Supplementation of Hong Kong Caterpillar (*Tenebrio molitor*) on Quail Egg Quality

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ABSTRACT

The Hong Kong caterpillar (HC) is an alternative source of animal protein for feed. This research aimed to study the effect of using Hong Kong caterpillars in the quail diet on egg quality. A total of 200 quail aged 8-14 weeks, weighing 110 ± 10 g, were used in the study, with 40% production. This study used a completely randomized design with five treatments and four replications. The laying quail diets were formulated with varying levels of HC, including 0% HC for group A, 3% HC for B, 6% HC for C, 9% HC for D, and 12% HC for E. The egg quality parameters measured were egg yolk fat, egg yolk cholesterol, egg white protein, and eggshell thickness. The results indicated that including 12% HC in the quail diet significantly reduced egg yolk cholesterol and egg yolk fat. However, eggshell thickness and egg white protein remained unaffected. Consequently, it can be concluded that Hong Kong caterpillars can be used in quail diets up to a maximum of 12% to reduce egg yolk cholesterol and fat while maintaining eggshell thickness and egg white protein levels.

Keywords: Egg quality, Fish meal, Hong Kong caterpillar, Quail

INTRODUCTION

Poultry farming is a promising business venture due to the growing demand for poultry eggs yearly. Eggs, including quail eggs, are a valuable source of animal protein that humans require and are relatively affordable compared to other animal protein sources, such as meat. Moreover, eggs are considered a complete source of nutrients, providing a balanced combination of essential amino acids, minerals, and vitamins (Saraswati and Tana, 2021).

Quail (*Coturnix coturnix japonica*) is cultivated for its eggs due to its high egg productivity, reaching 200-300 eggs/head/year (Amo et al., 2013). Quail eggs are foods with complete nutritional content that are helpful for the body, especially for children in their infancy. The nutritional content of quail eggs is 3-4 times greater than that of chicken eggs. Quail eggs have a nutrient composition that includes 13.30% crude protein, 11.99% crude fat, and 1993 kcal/kg metabolic energy (Thomas et al., 2016). In addition, they contain various amino acids, such as lysine 790 mg/100 g, valine 865 mg/100 g, and leucine 1139 mg/100 g, and fatty acids such as linoleic 2.58 g/100 g, oleic 8.84 g/100 g, palmitic 5.39 g/100 g, and stearate 2.03 g/100 g. However, quail eggs contain a high amount of egg yolk cholesterol (877.38 mg/100 g; Tunsaringkarn et al., 2013; Nuraini et al., 2020).

During 2016-2020, quail egg production increased from 23.575 tons to 24.205 tons (Statistics of Livestock and Animal Health, 2020). Quail eggs are in great demand among the public and producing high-quality quail eggs suitable for feed (Emeka Aba and Anayo Onah, 2015). Currently, fish meal is the primary source of animal protein used in artificial feed, as it has a high crude protein content of 64.27% (Praptiwi and Wahida, 2021). However, the availability of imported fish meals is still a challenge, and alternative sources of animal protein are needed (Nuraini et al., 2022). One alternative feed source of animal protein is the Hong Kong caterpillar (HC, *Tenebrio molitor*), which is the larvae of insects found in grain products with high nutritional content. The HC cultivation is relatively easy and has promising business opportunities due to increasing demand. Moreover, HC has a protein content based on the nutrient content of their culture media (Nuraini et al., 2022).

The nutritional content of HCs with a mixture of 50% concentrate culture medium and 50% tofu dregs fermented with microbes in Natura Organic Decomposers was 62.35% crude protein, 17.07% crude fat, 7.35% crude fiber, 0.23% calcium, 0.97% phosphorus, and 3998.31 kcal/kg metabolic energy (Nuraini et al., 2022). Hong Kong caterpillars contain high levels of amino acids, such as glutamic acid (6.86%), alanine (5.37%), aspartic acid (4.80%), lysine (4.75%), leucine (4.49%), valine (3.83%), glycine (3.40%), tyrosine (3.04%), and methionine (0.43%). They also contain high unsaturated fatty acid percentages, including 34.24% linoleic acid (omega-6), 21.28% oleic acid (omega-9), 2.45% stearic acid, 1.20% myristic acid, and 0.15% linolenic acid (omega-3), and saturated fatty acids, such as 10.04% palmitic acid (Nuraini et al., 2022). Hong Kong caterpillars contain 7.2% chitin in the larval phase, while it is 9.54% in the pupal phase, and 11.8% in the adult phase (Yu et al., 2021).

The use of *Tenebrio molitor* in the diet of laying hens as a replacement for meat bone meal up to 5% can lead to good production performance, including increased egg weight and improved egg quality with the production of omega-3 fatty acids (Rahmawati et al., 2022). Hong Kong caterpillars, as a source of animal protein, have a high crude protein content (62.35%), high lysine content (4.75%), and high linoleic acid content (34.24%, Nuraini et al., 2022). Hong Kong caterpillars have almost the same crude protein content as grade one fish meal, that is 64.27% (Praptiwi and Wahida, 2021), 5.0% amino acid lysine, and 8.30% linoleic fatty acid (Zahroh et al., 2015). This makes them a suitable replacement for imported fish meals in the diet of quail.

High linoleic acid in the diet can affect the cholesterol content of the egg yolk (Fukumitsu et al., 2013), as alpha-linoleic acid (ALA) reduces cholesterol synthesis (Fukumitsu et al., 2013). Cholesterol synthesis uses saturated fatty acids as raw materials, while unsaturated fatty acids are not used in cholesterol synthesis (Ye et al., 2011). Chitin compounds found in HC can also reduce the egg yolk fat content. Chitin is a high fiber content that binds with fat and cholesterol, inhibiting their absorption by the body (Ye et al., 2018). It has a positive impact on lowering egg yolk fat levels and is also more effective in fat absorption (Ambarwati, 2017). As more cholesterol is bound to chitin, the greater the reduction in the cholesterol content of quail eggs (Herdyastuti and Daniar, 2019).

Shell thickness is closely related to the levels of calcium and phosphorus in the diet. The calcium and phosphorus contents in HCs (0.19% and 0.82%, respectively), are lower than those in imported fish meal (3.10% calcium and 1.89% phosphorus; Nuraini et al., 2022). The effect of using HCs as a substitute for imported fish meal on egg yolk fat, egg yolk cholesterol, egg white protein, and eggshell thickness of quail eggs is still unknown. Therefore, the current study aimed to evaluate the effect of HCs as a replacement for imported fish meals on quail egg quality.

MATERIALS AND METHODS

Ethical approval

This research was approved by the Research Ethics Committee of the Faculty of Animal Science, Universitas Andalas Padang, Indonesia. Ethics study of experimental animals' guideline, according to law number 18 of the Republic of Indonesia (2009) about Animal livestock and animal husbandry.

Birds and housing

Quail were kept in a quail house at the Faculty of Animal Science, Universitas Andalas Sumatra Barat, Indonesia. The temperature of the quail house ranged 23-25°C with a humidity of 50-70%. Vaccination was administered to one-week-old quails by the breeders, and vaccination was carried out via drinking water when the quails reached 4 weeks old. The lighting on laying quail maintenance was given 15 hours a day.

The experimental birds used in this study were 200 quails (*Coturnix coturnix japonica*) in the 8-week-old layer phase (weight of 110-120 gram) with 40% egg production. Quail were reared for 6 weeks to evaluate the egg quality of the quail. The laying quails were purchased from quail breeders at the age of 3 weeks and reared up to 6 weeks of age with commercial rations. Following this, a two-week adaptation period to the treatment ration was conducted before commencing the actual treatment, which took place between the ages of 8 to 14 weeks. The experimental setup consisted of 20 wire cages, each measuring 60x50x50 cm and housing 10 quails per cage unit.

Hong Kong Caterpillar propagation

The culture medium for HCs consisted of Concentrate 126 production PT Charoen Phokp and tofu waste (1:1) that was sterilized in an autoclave, cooled to room temperature (27-30°C), inoculated with 1% Natura Organic Decomposer (1 ml of Natura Organic Decomposer with 100 ml of water and 2 g of sugar) and incubated for 4 days. After that, the fermented medium was dried and ground. Then, the medium was put into a biopon (up to 500 g), and 5 g of HCs were added and aged for 10 days. After 30 days, the HCs were harvested and sieved to separate them from the media. Finally, the HC was dried and ground into flour (Nuraini et al., 2022).

Treatment feed

The feed given to quail were self-mixed diets using feed ingredients, such as corn meal, soybean meal, imported fish meal, HC, fine bran, coconut oil, bone meal, and top mix. All the ingredients for the rations were finely ground and mixed into rations. The rations were given in mash form. The water was given *ad libitum*. The content of feed substances and metabolic energy of the ingredients for the treatment diets are shown in Table 1.

Research design

The current experimental method employed a completely randomized design with five treatments and four replications. The treatment diets used HCs in quail diets at percentages of 0% (A), 3% (B), 6% (C), 9% (D), and 12% HC (E). A total of 200 quails were divided into five groups (A, B, C, D, and E), with 40 quails per group. Each group was further subdivided into four replications denoted as A1, A2, A3, and A4, with each replication comprising of 10 quails. The diet was composed of iso-protein and iso-energy with a crude protein content of 20% and metabolic energy of 2800 kcal/kg. The composition of the feed ingredients of the treatment diets is shown in Table 2, and the contents of feed substances and metabolic energy of the treatment diets are presented in Table 3.

Measured parameters

The study measured several parameters related to egg quality. The fat content of the egg yolk was determined as a percentage using the Sokhlet method, according to the Association of Official Analytical Chemists (AOAC, 2016). The egg yolk cholesterol concentration was also measured and expressed as milligrams per 100 grams of egg using the Lieberman-Buchards method as described in a study by Sudarman et al. (2018). Additionally, the egg white protein content was measured as a percentage using the Kjeldahl method, which is a standard analytical procedure established by the AOAC (2016). Finally, the thickness of the eggshell was determined by breaking quail eggs and measuring the eggshell parts at the blunt end, middle, and pointed end using a scrub micrometer. The eggshell thickness was determined following the protocol described by Sudrajat et al. (2014).

Data analysis

All data obtained were statistically processed by SAS software (version 2003), and analysis of variance was done. Duncan's multiple range test was used to evaluate significant differences between treatments ($p < 0.05$).

Table 1. The feed ingredients and metabolic energy (kcal/kg) of the quail diet[a]

Nutrient Feed Ingredient (%)	**Crude protein**	**Fat**	**Crude fiber**	**Ca**	**P**	**EM (kcal/ kg)**[c]	**Lysine**	**Methionine**	**Glutamate**	**Linoleate**	**Chitin**
Corn meal	9.58	2.66	3.50	0.38	0.19	3300	0.20	-	2.28	2.20	-
Soybean meal	43.76	2.49	4.50	0.63	0.36	2240	2.60	0.50	8.15	0.81	-
Import ted fish meal	64.00	2.85	3.90	3.10	1.89	2540	5.00	0.99	7.30	8.30	-
Hong Kong caterpillar[b]	62.35	14.96	6.18	0.19	0.82	3362	4.75	0.43	6.86	34.24	7.20
Fine bran	12.34	5.09	14.50	0.69	0.26	1630	0.77	0.29	1.92	3.57	-
Coco nut oil	-	100.00	-	-	-	8600	-	-	-	1.92	-
Vitamin B12 [d]	-	-	-	49.00	14.00	-	-	-	-	-	-
Top mix [d]	-	-	-	0.06	-	-	-	-	-	-	-

[a] Nuraini et al. (2020); [b] Nuraini et al. (2022) [c] Scott et al. (1982); [d] Top Mix production of PT.

Table 2. Composition of the feed ingredients in each treatment diet

Feed Ingredient (%) \ Treatment Ration	A	B	C	D	E
Corn meal	52.75	52.75	52.75	52.75	52.75
Soybean meal	13.00	13.25	13.25	13.50	13.75
Fish flour	12.00	9.00	6.00	3.00	0.00
Hong Kong caterpillar	0.00	3.00	6.00	9.00	12.00
Fine bran	14.00	14.00	13.75	13.75	13.75
Coconut oil	2.75	2.50	2.25	2.00	1.75
Vitamin B12	5.00	5.00	5.50	5.50	5.50
Top mix	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00

Table 3. Feed substances and metabolic energy (kcal/kg) of each treatment diet

Nutrient (%) \ Ration	A 0% HC	B 3% HC	C 6% HC	D 9% HC	E 12% HC
Crude protein	20.15	20.21	20.13	20.19	20.25
Fat	5.53	5.65	5.75	5.87	5.99
Crude fiber	3.96	4.08	4.16	4.28	4.40
Calcium	3.20	3.12	3.27	3.19	3.10
Phosphorus	1.11	1.08	1.12	1.09	1.05
Lysine	1.15	1.15	1.14	1.14	1.14
Methionine	0.40	0.40	0.39	0.39	0.38
Glutamate	3.40	3.40	3.39	3.40	3.41
Linoleate	2.81	3.69	4.35	5.13	5.91
Chitin	0.00	0.22	0.43	0.65	0.86
Metabolic energy	2.801	2.810	2.809	2.818	2.826

Description: Calculated based on Tables 1 and 2

RESULTS AND DISCUSSION

Egg quality

Egg quality is a collection of egg characteristics that affect consumer tastes. Egg quality refers to several standards that determine both internal and external quality. The quality of internal eggs carried out in this study was the content of cholesterol, fat and protein, and shell thickness for external egg quality. The previous study, according to Nuraini et al. (2022), *Tenebrio molitor* caterpillar could be used up to 12% in laying quail rations (substituted 100% fish meal) and maintain the same egg production as control. Nuraini et al. (2020) found the quail egg yolk fat 27.98- 29.58% and the egg yolk cholesterol 711.00-877.38 mg/100 g when fed fermented cacao pod. Jajić et al. (2020) found unsaturated fatty acid content of *Tenebrio molitor* larvae is very high, that is oleic acid (C18:1) in 40.83%, linoleic acid (C18:2, omega-6 fatty acid) with 29.80% and linolenic acid (C18:3) with 1.08%. Nuraini et al. (2022) found the fatty acid content of *Tenebrio molitor* was 34.24% linoleic acid and 21.28% oleic acid. The longer the carbon chain and the greater the number of double bonds in an unsaturated fatty acid, the greater its tendency to lower cholesterol levels in eggs (Shramko et al., 2020). Ait-Kaki et al. (2022) have reported the egg cholesterol of laying hens decreases if fed *Tenebrio molitor* larvae and Turmeric powder (Curcuma). According to Secci (2021), found the egg shell thickness of quail 29.52 µm when fed *Tenebrio molitor* larvae meal replaced 20% protein of soybean. Table 4 tabulates the data on the quality of quail eggs when the quails' diet was supplemented with HCs for 6 weeks.

Egg yolk fat

Table 4 shows that the egg yolk fat of quail eggs was affected by HCs in the diet and ranged 31.47-36.09%. Moreover, the use of HCs up to 12% in the diet had a significant effect on quail egg yolk fat compared to other treatment groups ($p < 0.05$). The test results showed that quail egg yolk fat in treatment E (12% HC) was significantly lower than that in treatments D, C, B, and A ($p < 0.05$).

Compared to treatments D, C, B, and A, the lower quail egg yolk fat in treatment E was associated with decreased quail egg yolk cholesterol. The study indicated that the concentration of cholesterol in quail egg yolk decreased by 26.14% in treatment E, compared to other groups. This reduction in cholesterol content also led to a decrease in the fat content of the quail egg yolk since cholesterol is a lipid. Regarding the chemical structure of cholesterol, Yanagisawa et al. (2022) have reported that cholesterol is a complex lipid compound. The broader category of lipids includes consists of triglycerides (neutral fat), phospholipids, and cholesterol. According to Huff et al. (2022), fat is composed of various components, including cholesterol, pigments, and vitamins A, D, E, and K.

The chitin content also influenced the decrease in egg yolk fat in treatment E in the diet. The chitin content in treatment E was 0.86%. This amount is related to the chitin content of the HC, which is 7.2% in the larval stage (Yu et al., 2021). Chitin binds fat, and the bound fat is carried away in the feces (van den Broek and Boeriu, 2019). According to Zhen et al. (2022), chitin can bind fat (as well as cholesterol) to inhibit fat absorption by the body. The fat bound to chitin forms a compound that is not absorbed. The decrease in fat absorption is because chitin in the stomach is converted into a gel by gastric acid and then wraps cholesterol and fat molecules in gastric juice (Zhou et al., 2020).

Egg yolk cholesterol

The highest quail egg yolk cholesterol was recorded for treatment A (0% HC, 965.13 mg/100 g), and the lowest egg yolk cholesterol was seen in treatment E (12% HC, 712.88 mg/100 g). The analysis of variance showed that the use of HCs up to 12% in the quail diet had a significant effect on quail egg yolk cholesterol ($p < 0.05$), compared to another group. It was found that quail egg yolk cholesterol with treatment E was significantly lower than that with treatments D, C, B, and A ($p < 0.05$). Increasing the use of HCs could reduce the quail egg yolk cholesterol content. The high level of the unsaturated fatty acid linoleic acid caused the low cholesterol content in quail egg yolks with diet treatment E. The E treatment resulted in a linoleic fatty acid content of 5.91%, which was related to the high linoleic acid content in HCs. A recent study found that the fatty acid content of HCs was 34.24% linoleic acid and 21.28% oleic acid (Nuraini et al., 2022). Jajić et al. (2020) found unsaturated fatty acid content of Tenebrio molitor larvae is very high, that is oleic acid (C18:1) in 40.83%, linoleic acid (C18:2, omega-6 fatty acid) with 29.80% and linolenic acid (C18:3) with 1.08%. Linoleic unsaturated fatty acid is a fatty acid that contains double bonds (Coniglio et al., 2023). According to Shramko et al. (2020), the longer the carbon chain and the greater the number of double bonds in an unsaturated fatty acid, the greater its tendency to lower cholesterol levels. According to Azemi et al. (2023), linoleic acid is one of the foods shown to lower cholesterol levels in the blood. Research by Azemi (2023) showed that the linoleic acid content could also reduce cholesterol. The provision of a diet containing the unsaturated fatty acids linoleic acid and linolenic acid can lower cholesterol, triglyceride, and LDL levels and raise HDL levels (Dwiputra et al., 2015).

Furthermore, one factor that lowers blood cholesterol is replacing saturated fatty acids with polyunsaturated fatty acids (unsaturated fatty acids). A study by Fukumitsu et al. (2013) showed that alpha-linolenic acid (ALA) reduced cholesterol synthesis. According to Jajić et al. (2020), Tenebrio molitor has linoleic acid 28% and linolenic acid 1%. Cholesterol synthesis uses saturated fatty acids as raw materials, while unsaturated fatty acids are not used in cholesterol synthesis. Linolenic acid is an unsaturated fatty acid. Higher linolenic acid levels in the blood are not used for cholesterol synthesis, causing lower egg yolk cholesterol.

The chitin content had a significant effect on the reduction of the egg yolk cholesterol content in treatment E. The chitin content was 0.86% higher in treatment E than in the other treatments. The chitin content of HCs was reported to be 7.2% in the larval stage (Yu et al., 2021). According to Xu (2017), chitin has been shown to bind with bile acids and excrete them in the feces, resulting in lower cholesterol levels due to the requirement of bile acids to form cholesterol. Furthermore, feed containing chitin derived from shrimp heads can reduce duck egg cholesterol levels (Saty et al., 2014).

The quail egg yolk cholesterol level at 12 weeks using HCs in diets up to 12% was found to be 712.88 mg/100 g. This level is lower than that obtained by Nuraini et al. (2017), which measured quail egg yolk cholesterol levels at 20 weeks at 746.38 mg/100 g. The cholesterol content of quail egg yolks was also lower than that of quail egg yolks aged 7-13 weeks, which was reported to be 877.38 mg/100 g by Nuraini et al. (2020).

Egg white protein

In the present study, the use of HC (*Tenebrio molitor*) as a source of animal protein resulted in egg white protein levels in the range of 12.00-12.58%. The results of the analysis showed that HC had no significant effect on quail egg white protein ($p > 0.05$). The crude protein content in the diet from treatment A (0% HC) to treatment E (12% HC) ranged from 20.15%-20.25%, respectively. The protein content of the diet indicated that the protein quality of HCs was 62%, similar to that of fish meal grade one (64%). Using up to 12% HCs in the laying quail diet had a similar effect on quail egg white protein and could reduce the use of fish meal by 100%. According to Omidiwura et al. (2016), protein is an essential nutrient in the diet since it influences egg production and quality (Ardiansyah, 2016).

The protein content of quail egg whites obtained in this study was within the normal range of 12-12.58%. The protein content of egg white with 12% HC in the quail diet was 12.24%. The protein content of quail egg whites was lower than that obtained in the study by Djaelani (2017), who obtained a quail egg protein percentage of 13.10%.

Egg shell thickness

Table 4 shows that the quail eggshell thickness ranged from 0.27 to 0.29 mm. The dietary supplementation of HCs up to 12% had no significant effect on quail eggshell thickness ($p > 0.05$). The difference stems from the fact that eggshell thickness is closely related to the calcium and phosphorus contents in the diet. The calcium and phosphorus contents in the diet for each treatment were almost the same, ranging from 3.10-3.27% to 1.05-1.12%, respectively. Calcium and phosphorus are needed to form eggshells, and their availability in the blood determines eggshell formation (Rathnayaka et al., 2020).

The present study showed that the shell thickness of quail eggs using 12% HCs in the diet was between 0.27 and 0.29 mm. The results of the thick quail eggshell were higher than those reported in the study by Rondonuwu et al. (2018), in which the quail shell thickness ranged from 0.18 to 0.19 mm. The results of the current study were not much different from the thickness of the quail egg shells obtained by Rudini et al. (2020), which was 0.26-0.27 mm. Based on the results of Ergun and Yamak (2017), a suitable eggshell thickness for quail eggs ranges from 0.2 to 0.35 mm. Agboola et al. (2017) found the quail eggshell thickness to be 0.30 mm when the given ration included protein 20% and energy metabolism 3000 kcal/kg.

Table 4. Egg yolk fat, egg yolk cholesterol, egg white protein, and eggshell thickness of quail eggs (mean ± standard error)

Treatment	Egg yolk fat (%)	Egg yolk cholesterol (mg/100 g)	Egg white protein (%)	Eggshell thickness (mm)
A (0% HC)	$36.09^{a} \pm 0.76$	$965.13^{a} \pm 5.26$	12.00 ± 0.03	0.28 ± 0.02
B (3% HC)	$34.58^{ab} \pm 0.70$	$909.51^{b} \pm 5.02$	12.02 ± 0.02	0.27 ± 0.01
C (6% HC)	$33.56^{ab} \pm 0.74$	$874.75^{c} \pm 4.96$	12.56 ± 0.01	0.29 ± 0.02
D (9% HC)	$33.37^{ab} \pm 0.80$	$845.40^{d} \pm 5.13$	12.43 ± 0.02	0.28 ± 0.01
E (12% HC)	$31.47^{b} \pm 0.79$	$712.88^{e} \pm 5.16$	12.58 ± 0.04	0.28 ± 0.02

^{abcde} Different superscripts in the same column showed significant differences at level of $p < 0.05$.

CONCLUSION

In conclusion, the use of up to 12% HCs in the diet of laying quails can replace 100% of imported fish meal, reduce egg yolk fat and egg yok cholesterol, and maintain egg white protein and eggshell thickness. Under these conditions, the obtained egg yolk fat content was 31.47%, the egg yolk cholesterol content was 712.88 mg/100 g (a decrease of 26.14%), the egg white protein content was 12.58%, and the eggshell thickness was 0.28 mm. Therefore, future studies are suggested to investigate the dietary supplementation of HC in laying ducks to obtain the optimum level and to study the efficiency ratio to reduce egg yolk cholesterol of laying ducks.

DECLARATION

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Authors' contributions

Nuraini contributed to creating the research ideas, designing the experiments, analyzing the data, and writing this article. Ade Djulardi and Robi Amizar contributed to using HC in quail feed and reviewed the written paper. Yuliaty Shafan Nur and Yesi Chwenta Sari analyzed the data and revised the article. All authors checked and approved the final version of the manuscript for publication in the present journal.

Ethical consideration

The author has ensured that this article complies with the journal's ethical issues (including plagiarism, consent to publish, infringement, data falsification, double publication, and redundancy) for submission and publication.

Conflict of interests

No conflicts of interest come from all the authors.

Availability of data and materials

Materials and data are provided by the authors upon reasonable request.

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