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Nutritional characteristics of Konara (*Quercus serrata*) acorns and their parasitic weevil larva (*Curculio dentipes*) and its palatability to Japanese black bears (*Ursus thibetanus*) as a food source in autumn

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ABSTRACT

We investigated the chemical components and energy content of Konara (Quercus serrata) acorns and their parasitic larval insect (Curculio dentipes). We then fed acorns to Japanese black bears (Ursus thibetanus) in captivity and monitored their eating behavior to evaluate the palatability of acorn cotyledons, parasitic larvae, and larval feces. We purchased 70kg of fresh acorns of Q. serrate and randomly selected 2512 of them and found that 17.7% of seeds were attacked by weevil larvae. The contents of protein and fat in the larval body were 28.6% and 53.7%, respectively. The gross energy of the larval body was 29.6kJ/DM g and the energy value was 1.8 times higher than that of acorn embryos. Our results suggest that the consumption of larvae parasitizing the acorns of Q. serrate contributes to the acquisition of animal protein and energy under incidental predation by large post-dispersal acorn consumers, such as deer or wild boar. In addition, in our feeding experiment, bears discarded acorns filled with larval feces but showed much interest in living larvae and quickly consumed them finding them. This eating behavior shows that weevil larvae are highly palatable to bears, which may acquire animal protein and energy from them.

Key words: Acorn, Cotyledons, Protein, *Quercus serrata*, *Ursus thibetanus*, Weevil

INTRODUCTION

The weevil larva, *Curculio dentipes*, is generally found in the acorns of deciduous broadleaved trees, especially Konara (*Quercus serrata)* trees, in Japan [1]. During acorn development and maturation, an adult female weevil perforates the seed coat with her snout and oviposits into the acorn embryos (cotyledons) [2]. After hatching, the larvae consume acorn embryos and grow inside the pericarp (seed wall). Because a single acorn may be attacked by the different female insects, multiple larva may be deposited in the same acorn. Once sufficiently mature, larvae make holes in the pericarp and exit the acorn. Feces excreted by the larvae remain inside the pericarp. An incidental predator will have an opportunity to take acorns with or without larvae inside them. When wild animals and birds obtain acorns containing larvae inside the pericarp, they may receive additional nutrients of larval insect origin instead of acorn embryos. However, the nutritional contribution of weevil larvae has not been considered as a wildlife food source in autumn. The chemical composition of larval feces and whether acorn predators will consume larval feces remaining in the acorn pericarp remain unknown.

It is well-known that *Quercus* species have a mast-seeding habit meaning that the annual acorn production is not stable [3-5]. For *Q. serrata* in Japanese beech tree forests, good crop years are generally cyclical at an interval of two or three years. Although this cyclical acorn production is considered a useful strategy for mother trees to survive, poor crop years result in less opportunity for weevil mother insects to oviposit in acorns and also lead to a shortage of food resources for wildlife. Although this acorn-insect-wild life relationship has independently been studied by plant scientists, entomologists, and wildlife researchers, no integrated reports or data describe the relationship between food sources and nutritional consumption by wildlife in this habitat. For instance, most wildlife researchers have observed that Japanese black bears, deers, and wild boars consume the acorns of beech trees in autumn, however, they have ignored not only the nutritive characteristics (or value) of acorns but also the contribution of living larvae as a wildlife food source.

In this study we aim to compare the chemical components and energy density of the acorn embryos of *Q. serrata*, weevil larvae (*C. dentipes*), and larval feces remaining in the acorn pericarp. We also monitored the feeding response (eating behavior) of Japanese black bears (*Ursus thibetanus*) to evaluate the selectivity (palatability) of acorns and weevil larvae.

MATERIALS AND METHODS

Preparation of acorns and larvae

Seventy kilograms of fresh Konara (*Quercus serrata*) acorns were purchased from the Gunma Seed and Sapling Association (Numata-shi, Gunma, Japan). The acorns were divided into several paper boxes and stored for a few days at room temperature. We then collected larvae that had exited the shell and stored them in a plastic bottle at -20℃. At the same time, the acorns were randomly selected from each box and a total of 2512 acorns were used to characterize damage to the acorn embryos by weevil larvae. Damage evaluation was performed by cutting the acorn with a scalpel and inspecting the acorn embryos (cotyledons). Normal acorn pericarps and larval feces were also collected from the acorn and stored in plastic bags at -20℃ until analysis. All samples (acorn embryos, larvae and larval feces) were freeze-dried in a freeze dryer (EYELA, FDU-2100; Tokyo Rikakikai Co. Ltd., Japan) for a few days and then plant organs (acorn embryos) were ground in a Wiley mill to pass a 1mm screen and stored in sealed bottles. The moisture content of the samples was calculated by differences in weight after freeze-drying.

Chemical analysis and measurement of gross energy content

Ground sample of the acorn embryos, larval body (whole body), and larval feces were used for crude protein and fat analysis by the Kjeldahl method and ether-extract procedures, respectively, according to the AOAC [6]. The gross energy content of all samples was measured with a bomb calorie-meter (IKA C-5000; IKA Japan, Co. Ltd.).

Observation of selectivity of acorn and larvae by Japanese black bears

A feeding experiment to observe the eating behavior of bears was performed at the Ani Bear Farm (Kitaakita-shi, Akita). Three adult male Japanese black bears (average body weight 85.3kg) were used. They were kept separately in each pen (experimental plot) and fed a standard diet (Oriental Co. Ltd.) through the experimental period. Before feeding in the morning, approximately 100g of acorns randomly selected from the paper boxes was placed on the feed tray and then, an observer monitored the eating behavior of the bears. The observation was continued until the bear finished eating the acorns and three repetitions were performed for each bear.

Animals were cared for throughout the experimental period according to the Cruelty to Animal Act for the Care and Use of Exhibition Animals (Shirei-Dou-49-3).

Statistical analyses

Data were subjected to analysis of variance and statistically significant differences were determined by the Student's t-test. The differences between mean values were tested using the least significant difference method at a 5% level of significance [7].

RESULTS AND DISCUSSION

Morphological observation of acorn embryos attacked by weevil larvae

Of the 2512 acorns of *Q. serrate* used for damage assessment, 17.7% were attacked by weevil larvae (Fig. 1.).

Although the extent of damage was slightly different in each acorn embryo, it seemed to impede germination. In typical cases of damage, almost all embryos (cotyledons) were eaten by the multiple larvae. In addition, living larvae remained inside the seed shell (pericarp) with their feces (Fig. 2.).

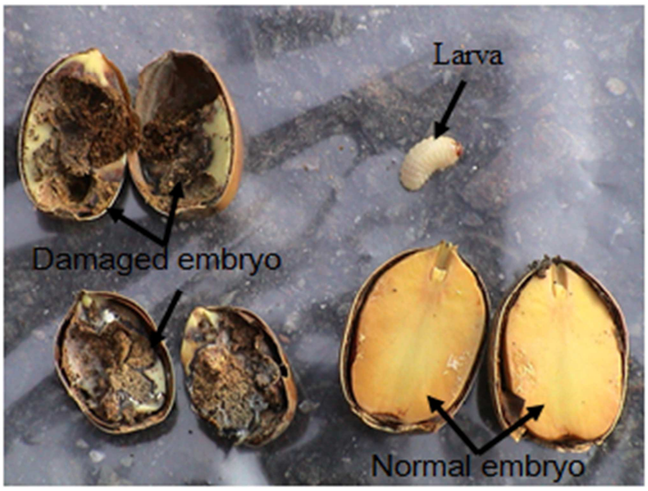

Figure 1. Photographic images of normal and damaged acorn embryo (cotyledons) and parasitic larva.

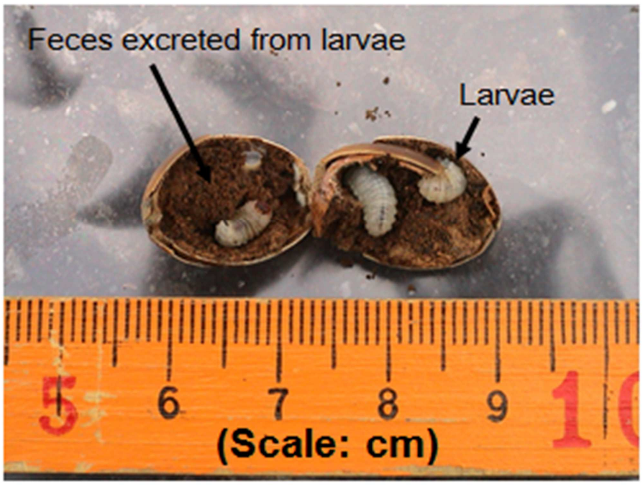

Figure 2. Interior appearance of acorns attacked by parasitic insects (*Curculio dentipes*) and living larvae.

In contrast, 11.8% of the normal acorns that escaped parasitic (weevil) attack sprouted radicles from the shell.

Tani [1] reported that the parasitism rate of *Q. serrata* acorns by weevil larvae ranged from 9.9% to 50.5% in four secondary-forests in Kanagawa, Japan and suggested that the extent of parasitism was related to the mast-seeding cycle. The higher the acorn production, the lower was the parasitic rate in a given year. In our experiment, acorn samples were obtained from a seed dealer, but acorn production was good in the producing area. The weights and sizes used in the experiment were similar to those in previous studies. Our data thus show a lower rate of parasitism by weevil larvae.

Tani [1] also reported that the number and size (fresh weight) of weevil larvae per acorn ranged from 1.6 to 2.5 and from 35.6 to 51.6mg, respectively. Larval weight tended to increase with altitude, considering that larval weight in the acorns from trees growing in plains were greater than those from trees on mountain sides. Bonal and Munoz [8] investigated the relationship between the number of weevil larva (*Curculio* spp.) and the larval size in Holm oak (*Q. ilex*) acorn and found that the larval size increased with date and with size of infested acorns but declined with higher conspecific competition provoked by larger numbers of larvae per acorn. In our experiment, we did not measure the number and weight of larvae, but when inspecting embryos, observed one to four larvae per acorn, all of which were still alive. Weevil larvae generally remain approximately 20 days in the acorn pericarp before exiting. Desouhant *et al*. [2] reported that the larval mortality outside the acorn was very low, and Bonal and Munoz [8] also reported that the larval mortality of infesting the *Q. ilex* acorns was less than 1%.

Schroder *et al.* [9] reported that lesions in the acorns of *Q. robur* were caused by the infection of some fungus. In the present study, we did not classify and test the acorns from this point of view. Moreover, because our experimental period was short, fungus could not grow enough from the cotyledon.

Chemical composition and energy content of samples
The chemical composition and energy content of the acorn embryos, larvae, and larval feces are presented in Table 1.

Table 1. Chemical composition of mature acorns of *Quercus serrata* and larvae of *Curculio dentipes*

	Crude protein (DM %)	Crude fat (DM %)	Gross energy (kJ/DM g)
Quercus serrata			
Acorn (whole)	4.1 ^{a}	1.5 ^{a}	16.8^{a}
Embryo	4.7^{a}	2.2^{a}	16.4^{a}
Pericarp	2.3^{b}	0.2^{b}	18.2^{a}
Curculio dentipes			
Larva whole body	28.6^{c}	53.7^{c}	29.6^{b}
Larval feces	4.5^{a}	0.9^{a}	15.7^{a}

a, b, c) Values with different superscript letters in a row are significantly different ($p<0.05$).

The crude protein and fat contents of acorn embryos were 4.7% and 2.2%, respectively. The gross energy content was 16.4 kJ/g on a dry matter basis. Although there have been few studies regarding the chemical composition and energy content of *Quercus* acorns, these chemical parameters were similar to those of *Q. serrata* acorns collected in Okutama, western Tokyo [10].

The crude protein and fat contents of larval bodies were 28.6% and 53.7%, respectively, 6.1 to 24.4 times higher than those of the acorn embryos. The gross energy content of weevil larva was 29.6 kJ/g on a dry matter basis, 1.8 times higher than that of embryos. These results appear to be novel, considering that most researchers have focused on the development of acorn and the ecology of weevil larvae. Some of them have noted that the acorn embryo was a good food source for larvae living in the pericarp, but none have investigated the chemical composition and nutritive value of weevil larvae. Therefore, we cannot compare these results with those of other studies.

In the larval feces remaining in the acorn pericarp, the protein and energy content but not the crude fat content were similar to those of the acorn embryos.

Feeding manner and selectivity of Japanese black bears for acorn and larvae
In our feeding experiment, bears approached the feed tray and sat facing the tray. A bear carried an acorn to the mouth by picking it up with the hallux and second toe of the forefoot or with the tip of the tongue and masticated the acorn pericarp with its molars. The bear then pushed pieces of acorn out of its mouth onto the palm or the back of the fore-leg. At that point, if the acorn embryos were normal (not infested by weevil larvae), the bear ate the

embryos without consuming the pericarp (seed shell). Bears discarded the acorns whose pericarps were full of larval feces, but showed much interest in living larvae and quickly consumed them. This eating response followed them to acquire animal protein and energy from weevil larvae.

Tani [1] reported that the average number of larvae living in a *Q. serrata* acorn was 2.1 per acorn and that larval weights ranged from 20 to 30mg or from 50 to 70mg. The difference of the larval weight was considered to reflect the volume of embryos in the acorn, considering that weevil larvae eat embryos as their food source. Bonal and Munoz [8] also showed a relationship between the size of infested acorns and the weight of weevil larva (*C. elephas)*, a specialist predator of the Holm oak (*Q. ilex*). The relationship was logarithmic and the mean number of larvae per acorn was 2.3.

CONCLUSION

This study showed the chemical composition and energy content of weevil larvae (*C. dentipes*) and their feces compared with those of the acorn (*Q. serrata*) embryos. The protein and gross energy contents of the larval bodies were higher by 6 and 1.8 times, respectively, than those of acorn embryos. In practice, larvae living in the pericarp showed high palatability to Japanese black bear. Our results showed that weevil larvae contribute animal protein and energy as a wildlife food source.

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