会 報 目 次

(Vol. 61, No. 2)

Contents

(Vol. 61, No. 2)

平成29年度

家畜栄養生理研究会 秋季集談会開催のご案内

開催日時：2017年9月8日（金）

8：30～ 9：00　評議員会　（講義棟 26番講義室）

9：30～12：00　集談会　（講義棟 26番講義室）

12：30～13：30　意見交換会（生協食堂）

会　　場：信州大学伊那キャンパス

（〒399-4598 長野県上伊那郡南箕輪村8304

http://www.shinshu-u.ac.jp/guidance/maps/map07.html）

会　　費：集 談 会…… 一般会員 無料

非 会 員 2,000円

意見交換会 …… 一般会員 2,000円

（予定）

連 絡 先：北海道大学大学院農学研究院動物機能栄養学研究室

家畜栄養生理研究会（事務局庶務担当：小池 聡）

TEL & FAX：011-706-2812

E-mail：eiyoseiri2011@gmail.com

HP：http://www.agri.tohoku.ac.jp/ruminol/eiyoseiri.html

平成29年度

家畜栄養生理研究会　秋季集談会予定時間

9：40～10：15

Vishwajit Chowdhury（九州大学大学院生物資源環境科学府）
「L-Citrulline and L-leucine-mediated thermoregulation affords thermotolerance」

10：15～10：50

Htun Myint（北海道大学大学院農学院）
「Bean husk, an agricultural by-product having functionality in animal nutrition and health」

10：50～11：25

鈴木 知之（国際農林水産業研究センター）
「日本および東南アジアにおけるウシからの消化管発酵由来メタン排出抑制の可能性」

11：25～12：00

Seongjin Oh（北海道大学大学院農学院）
「Potency of ginkgo fruit for modulation of rumen microbiota and fermentation」

12：30～13：30　意見交換会（信州大学伊那キャンパス 生協食堂）

L-Citrulline and L-leucine-mediated thermoregulation affords thermotolerance

Vishwajit S. Chowdhury [1*], Guofeng Han [2], Hui Yang [2], Hiromi Ikeda [2], Takashi Bungo [3] and Mitsuhiro Furuse [2]

([1] *Graduate School of Bioresource and Bioenvironmental Science, Division for Experimental Natural Science, Faculty of Arts and Science, Kyushu University*; [2] *Graduate School of Bioresource and Bioenvironmental Science, Kyushu University*; [3] *Graduate School of Biosphere Science, Hiroshima University*)

1. Introduction

Thermal stress associated with global warming is a rising concern for the wellbeing of animals [1, 2]. Thermal stress in commercial chickens is a serious issue because high productive chickens are more sensitive to thermal stress compared with low productive native chickens. Commercial chickens can invest less energy for maintenance of their body temperature under thermal stress compared to native chickens [3]. Therefore, the body temperature of commercial chickens rises quickly under high ambient temperature (HT) [4-6], which may cause heat stress [7, 8]. Heat stress decreases food intake, live weight gain, and food efficiency in broilers [9], and affects egg production in laying hens [10].

Essential amino acids have been suggested as important nutrients to overcome heat stress problems in chicken husbandry [11-14]. Moreover, non-essential amino acids may also reduce the effects of psychological and physiological stress in chicks [15, 16]. It was found that the levels of free amino acids were modified by heat stress [17-19]. Plasma L-citrulline (L-Cit) concentrations declined in young chicks when they were exposed to HT (35°C for 48 h) [17]. L-Cit was first encountered as a constituent of watermelon (*Citrullus vulgaris*) [20, 21]. Watermelon is a good source of L-Cit as the dietary non-essential amino acid [22, 23]. L-Cit, which has physiological functions in most living systems [24], is well known to enhance the synthesis of L-arginine (L-Arg), the endogenous substrate for the production of nitric oxide (NO), and ultimately to increase endogenous NO production. The enzyme, NO synthase (NOS), converts L-Arg to NO. It has been suggested that NO influences the thermoregulation [26, 27]. However, until recently it was still not known whether L-Cit possesses any thermoregulatory functions and NO is involved in the L-Cit-mediated thermoregulation process. Moreover, it was also unknown whether L-Cit can afford thermotolerance in any species.

Thermal manipulation (TM) during embryogenesis is carried out through increasing the incubation temperature, which results in the acquisition of thermotolerance by neonatal chicks [28-30] and chickens [31] under HT. TM also has a long-lasting effect on energy balance that leads to an improved feed-conversion ratio [32]. Exposure to a daily cyclic higher temperature during embryonic day (ED) 10 to ED 18 decreased body temperature on the day of hatching (DOH) and improved thermotolerance in broiler [33, 34] and layer chicks [35] after hatching. It has been suggested that metabolic activities, especially energy metabolism, were significantly altered by TM [31, 36]. However, to the best of our knowledge, there was no report available on amino acid metabolism during TM in any species. Recently, we

Proceedings of Japanese Society for Animal Nutrition and Metabolism 61 (2):1-11, 2017.
*Correspondence to:
Vishwajit S. Chowdhury, PhD
Faculty of Arts and Science, Kyushu University, Fukuoka 819-0395, Japan
Tel & Fax: 092-642-2954
Email: vc-sur@artsci.kyushu-u.ac.jp

found that embryonic brain and liver content of leucine (Leu) was significantly declined by TM [37]. Therefore, the declined Leu raised some possibilities of its function in connection with body temperature regulation and thermotolerance.

In this article, we describe the thermoregulatory functions of L-Cit and L-Leu and their influence on thermotolerance. All the chick studies described in this article were performed in accordance with the guidelines for animal experiments carried out in the Faculty of Agriculture and on the Graduate Course of Kyushu University and adhered to Law no. 105 and Notification no. 6 of the government. The experiments using marketing age broilers were conducted following the regulation of the Animal Experiment Committee of Hiroshima University.

2. L-Cit-mediated thermoregulation and thermotolerance

To examine the thermoregulatory function of L-Cit, male layer chicks (Julia) (*Gallus gallus domesticus*) were purchased from a local hatchery (Murata hatchery, Fukuoka, Japan) and housed in wire-meshed cages in a group (20 birds per cage) at a constant temperature of 30 ± 1°C and with continuous lighting. After the acclimation period, a total number of 36 chicks were selected and divided into four groups again on the day of the experiment based on their initial rectal temperature in order to produce uniform groups (Experiment 1). The amino acids (L-Cit, L-Arg and L-ornithine (L-Orn)) were administered into the left ventricle of the chicks by intracerebroventricular (i.c.v.) injection via a microsyringe, according to the method described elsewhere [38]. The dose of the amino acids for i.c.v. injection was based on the findings of previous reports [15, 16]. In brief, chicks (5 days old) were intracerebroventricularly injected either with 1 μmol/10 μl of the above-mentioned amino acids or, for the control, with the same volume of saline solution. This injection method is not stress-causing as described by Furuse et al. [39] and Koutoku et al. [40]. In Experiment 2, 36 chicks were selected and divided into four groups as described in Experiment 1. Chicks (6 days old) were subjected to oral administration with amino acid solutions (L-Cit, L-Arg or L-Orn) using an elastic-plastic needle on a small syringe or, for the control, with 0.25% methyl cellulose solution. Chicks received the amino acids (L-Cit, L-Arg or L-Orn) by oral injection at a dose of 15 mmol/10 ml/kg body weight, based on our previous findings [16]. In Experiment 3, 28 chicks (6 days old) received three doses of L-Cit (3.75, 7.5 or 15 mmol/10 ml/kg body weight) by oral administration. Rectal temperature was recorded at 0, 30, 60, 90 and 120 min after i.c.v. or oral injections. In Experiment 4, the effect of L-Cit on thermotolerance was conducted with a total of 32 chicks (14 days old). Since our preliminary study found that a single (15 mmol/10 ml/kg body weight) oral administration of L-Cit was not able to provide thermotolerance, chicks were subjected to a dual oral administration of L-Cit (15 mmol/10 ml/kg body weight/administration) prior to being exposed to HT. After the first oral administration of L-Cit, chicks were allowed to remain at room temperature for 60 min. This was followed by a second administration and the chicks were then kept at room temperature for another 30 min before being exposed to one of two temperature-controlled chambers (Sanyo Electric Co. Ltd., Japan)—either HT (35 ± 1°C) or control thermoneutral temperature (CT: 30 ± 1°C)—for 180 min. Rectal temperature was measured at 0, 60, 120 and 180 min after being placed in the temperature-controlled chambers. Finally, Experiment 5 was conducted to examine the effect of L-Cit on thermoregulation and thermotolerance in marketing aged broilers. Chunky broilers were purchased (Fukuda hatchery, Okayama, Japan) and reared until 30 days old under optimum conditions of food, water, lighting, and temperature. Each broiler (about 2 kg body weight) was subjected to dual oral administration of L-Cit (20 mmol/10 ml/broiler) and followed the same process as described above in Experiment 4 before exposing to temperature controlled chamber (Matterhorn incubator, Japan) —either HT (30 ± 1°C) or CT (23 ± 1°C) for 120 min. Rectal temperature was measured at 0, 60 and 120 min after being placed in the temperature-controlled chambers.

The rectal temperature of chicks was measured with a digital thermometer with an accuracy of ±0.1°C (Thermalert TH-5, Physitemp Instruments Inc., USA) by inserting the thermistor probe into the rectum to a

depth of around 2 cm. For broilers, rectal temperature was measured by thermo recorder (TR-7s, T&D Corporation, Nagano, Japan). For the chick experiment, L-Cit, L-Arg, and L-Orn were purchased from Wako Pure Chemical Industries (Osaka, Japan). A large amount of L-Cit was bought for broiler experiment from Kyowa Hakko Bio (Yamaguchi, Japan). For i.c.v. injection, these amino acids were dissolved in 0.85% saline containing 0.1% Evans Blue solution. The solutions were dissolved by stirring them on a vortex, and they were sonicated in a bath sonicator for 10 min. The resulting dissolved amino acids were kept on ice during the i.c.v. injection. For oral administrations, the same amino acids were suspended in 0.25% methyl cellulose solution, and these suspensions were stirred well on a vortex. These solutions were kept at room temperature (30 ± 1°C) during the oral administration. The data for the rectal temperature derived from chick study were statistically analyzed by a paired *t*-test between 0 min and each time point after injection. A repeated-measure three-way ANOVA was conducted in Experiments 4 and 5 for analysis of body temperature. Significant differences were denoted as $P<0.05$. Data were analyzed using the statistical program Statview Version 5.0 (SAS Institute, Cary, USA, 1998). Values are presented as means ± S.E.M.

Rectal temperatures of chicks were not changed significantly between 0 min and each time point after i.c.v. administration of L-Cit, L-Arg, or L-Orn (Fig. 1) . Fig. 2 shows the changes in rectal temperatures following oral administration of L-Cit, L-Arg and L-Orn. It was found that L-Cit significantly ($P<0.05$) decreased the rectal temperature at 60 min. Conversely, L-Arg administration caused a significant ($P<0.05$) increment in rectal temperature at 90 and 120 min and L-Orn administration at 60 min compared with 0 min. The effect of different doses of orally administered L-Cit on rectal temperature during 120 min of the experimental period is shown in Fig. 3 . The highest dose of L-Cit caused to decrease the rectal temperature significantly ($P<0.05$) at 30, 60 and 120 min. Rectal temperature was increased significantly ($P<0.0001$) in chicks by HT (*data not shown*). Besides lowering rectal temperature under CT, L-Cit oral administration caused a significant ($P<0.0001$) reduction in the rectal temperature in heat-exposed chicks comparable to non-heat-exposed control chicks. A significant ($P<0.005$) interaction was found between HT and time, which indicates that the difference in rectal temperature increased with the progress

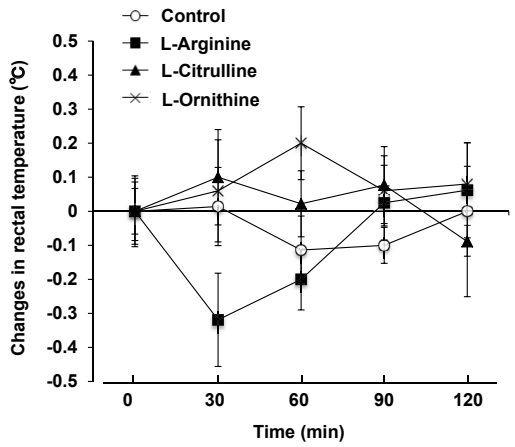

Fig. 1. Effects of central injection of L-citrulline, L-arginine or L-ornithine on rectal temperatures in chicks during 120 min of the experimental period. The number of chicks used in each group ranged between 5–9. Values are means ± S.E.M. Reproduced from Chowdhury VS, Shigemura A, Erwan E, Ito K, Bahry MA, Tran PV, Furuse M. 2015. Oral administration of L-citrulline, but not L-arginine or L-ornithine, acts as a hypothermic agent in chicks. J. Poult. Sci., 52:331–335, with the permission from Japan Poultry Science Association.

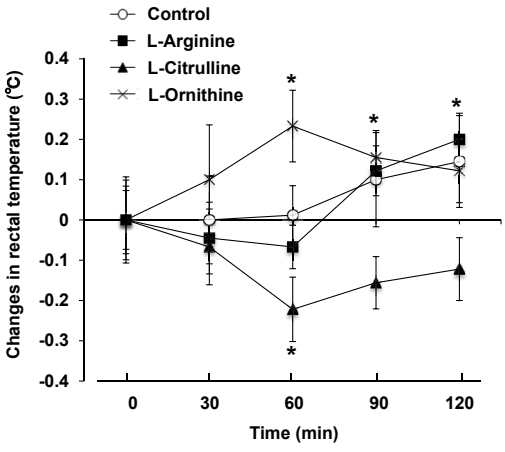

Fig. 2. Effects of oral administration of L-citrulline, L-arginine or L-ornithine on rectal temperatures in chicks during 120 min of the experimental period. The number of chicks used in each group was 9. Values are means ± S.E.M. *, $P<0.05$ vs. 0 min, by paired *t*-test. Reproduced from Chowdhury VS, Shigemura A, Erwan E, Ito K, Bahry MA, Tran PV, Furuse M. 2015. Oral administration of L-citrulline, but not L-arginine or L-ornithine, acts as a hypothermic agent in chicks. J. Poult. Sci., 52:331–335, with the permission from Japan Poultry Science Association.

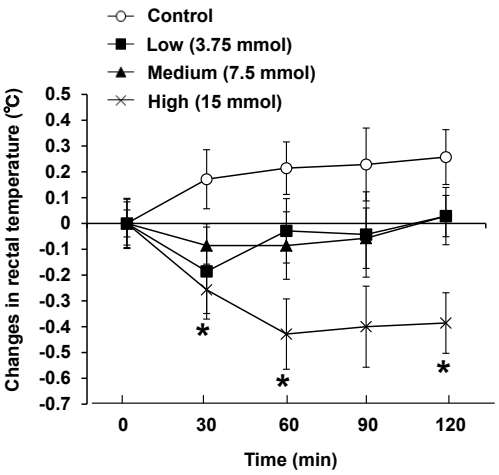

Fig. 3. Effects of different doses of orally administered L-citrulline on rectal temperatures in chicks during 120 min of the experimental period. The number of chicks used in each group was 7. Values are means ± S.E.M. *, P<0.05 vs. 0 min, by paired *t*-test. Reproduced from Chowdhury VS, Shigemura A, Erwan E, Ito K, Bahry MA, Tran PV, Furuse M. 2015. Oral administration of L-citrulline, but not L-arginine or L-ornithine, acts as a hypothermic agent in chicks. J. Poult. Sci., 52:331–335, with the permission from Japan Poultry Science Association.

of time under HT. HT also caused to increase rectal temperature (P<0.0001) in broilers (*data not shown*). In addition, L-Cit caused a significant (P<0.0001) reduction in the rectal temperature in heat-exposed broilers (*data not shown*).

The objective of the study was to reveal whether the amino acids (L-Cit, L-Arg and L-Orn) have thermoregulatory functions in chicks. Oral, but not central, administration of these amino acids altered rectal temperature. Oral administration of L-Cit prominently depressed rectal temperature, and conversely L-Arg and L-Orn slightly increased it. It was found that a single dose of i.c.v. injection of L-Cit, L-Arg or L-Orn did not cause any change in rectal temperature. Since we have used only a single dose for i.c.v. injection, we cannot preclude the possibility of the central effect of the three amino acids on the thermal center of the brain in thermoregulation.

Almost all of the L-Arg coming from the food supply is withdrawn from the portal blood by the liver to convert it to urea in mammals [24]. On the other hand, L-Cit can bypass the liver, since the liver is unable to uptake L-Cit from the portal circulation [41]. This bypassed L-Cit is converted to L-Arg by the kidney and released into the blood to make it available for the whole body. However, birds are lacking carbamyl phosphate synthetase, one of the enzymes of the urea cycle necessary for the synthesis of L-Cit from L-Orn [42]. Hence, birds cannot synthesize L-Cit or L-Arg from L-Orn but can synthesize L-Orn from L-Arg [43]. L-Orn is transported to the mitochondria after being synthesized in the cytosol. It is well known that mitochondria are important organelles in the cell which are involved in heat production and energy generation [44]. However, still it is unknown whether L-Orn has any involvement in the process of thermogenesis in the mitochondria. NO, produced in the conversion of L-Arg to L-Cit by the enzyme NOS [45], may play some roles as a hypothermic agent in chicks because thermoregulation has been proposed as one of the main physiological functions of NO [46]. However, our recent study found that NO may not be the main factor in L-Cit dependent hypothermia and thermotolerance (*unpublished*). Therefore, L-Cit itself and/or its metabolites or the altered metabolic functions induced by L-Cit may be involved in the process of hypothermia through influencing the thermal center of the brain and/or peripheral tissues. Therefore, further research on the determination of the concentration of these amino acids and their metabolites in the central and peripheral tissues as well as in the plasma would reveal the functional mechanisms of hypothermia induced by L-Cit. Compared with the other two lower doses, the highest dose (15 mmol/kg body weight for chicks or 20 mmol for broilers) was effective doses for inducing the hypothermic action. Our recent findings showed that plasma of chicks contains on an average 32.2 to 35.4 pmol/μl L-Cit [17]. Therefore, the used doses of L-Cit are pharmacological doses. Rimando and Perkins-Veazie [22] reported that watermelon rind contained more L-Cit than flesh on a dry weight basis which indicates that rind, an underutilized agricultural waste, could be used as a natural source of L-Cit to reduce high body temperature under HT. Future research on watermelon rind will clarify this hypothesis and may open further opportunity to find a natural source of L-Cit to fight against heat stress problems in chickens.

It was interesting to reveal that the L-Cit, which was a hypothermic agent in young chicks [47], did also

show the similar effects in marketing aged broilers. L-Cit-dependent thermotolerance in broilers would open new opportunity to examine its application to protect summer time rising of body temperature in the farms. Since L-Cit has already been reported as a strong antioxidant [48], it could be speculated that the utilization of L-Cit in poultry industry would be beneficial to protect heat stress related problems.

3. L-Leu-mediated thermoregulation and thermotolerance

To understand the involvement of amino acids in TM, free amino acids were analyzed in thermal manipulated broiler embryos. Experiments were conducted with Chunky broiler eggs. Fertilized eggs purchased from a local hatchery (Mori Hatchery, Fukuoka, Japan) were individually weighed, and placed in the incubators (Showa P008 type incubator, Showa Furanki Company, Saitama, Japan). Eggs were grouped on the basis of their weight to produce uniformity among the groups and were marked with a soft lead pencil for identification. Incubators had been set up with a temperature of 37.6°C and approximately 60% relative humidity. All eggs were candled on ED 7 and auto turned every h until ED 18. Eggs were transferred onto hatching trays from ED 19 and the undeveloped eggs and dead embryos had been removed. After hatching, day-old broiler chicks were housed in groups in metal cages at a constant temperature of 30 ± 1°C under continuous lighting until they were 4 days old. The temperature of the room was decreased to 28 ± 1°C once the chicks were 5 days old. Food (Adjust diets (metabolizable energy: >12.55 MJ/kg, protein: >23%)); Toyohashi Feed and Mills Co. Ltd., Aichi, Japan) and water were provided *ad libitum*.

In Experiment 1, 60 eggs were distributed into two incubators and set as two groups – namely, control stable temperature (ST) and TM. The ST group was incubated as described above, while the incubation temperature of the TM group was increased from 37.6°C to 38.6°C daily for 6 h (10:00 AM–4:00 PM) from ED 10 to 18 as reported by Yalçın et al. [49]. Developing embryos (n = 8–10) were randomly selected from each group and euthanized by cervical dislocation at ED 14 and 19. The euthanization of chicks at DOH was carried out using anesthesia with isoflurane (Mylan Inc., Tokyo, Japan). The brain and liver were collected and rapidly frozen in liquid nitrogen and then stored at −80°C until they were analyzed for free amino acid concentrations. Since the brain is the center of thermoregulation [50] and the liver plays a critical role in amino acid metabolism [51], we collected these tissues for amino acid analysis.

In Experiment 2, 50 eggs were randomly divided into five groups (n = 10 eggs) as follows: control (sterile water injected); L-Leu; L-isoleucine (L-Ile); L-valine (L-Val); and combined branched-chain amino acids (BCAAs: L-Leu + L-Ile + L-Val). Amino acids were dissolved into sterile water with doses of 35, 21 and 29 μmol/500 μl/egg for L-Leu, L-Ile and L-Val, respectively, as suggested by [52], and the combined BCAAs group was the combination of the above doses. In the preliminary study, we confirmed that there was no effect of injection of 500 μl of sterile water compared to no manipulation of eggs (no whole and no injection) on body temperature at hatching. The *in ovo* injection was performed on ED 7 following the method described elsewhere [53]. In brief, the large end of the egg (the injection site) was sterilized with 70% ethanol prior to a small hole being drilled in it. Sterile water or amino acid solution was injected into the yolk sac to a depth of 2.5 cm with a 1-ml disposable syringe that had a 25-gauge needle. The small holes at the large end of eggs were sealed immediately with Scotch tape upon completion of the injection. After completing the injection process, the eggs were returned to the incubator for incubation under ST. On DOH, time until hatching, hatchability, body weight and rectal temperature were recorded at 2 h post- hatching. Rectal temperature was recorded by a digital thermometer with an accuracy of ±0.1°C (Thermalert TH-5, Physitemp Instruments Inc., USA) by inserting the thermistor probe into the colon (rectum) through the cloaca to a depth of 2 cm.

In Experiment 3, 100 broiler eggs were randomly divided into two groups (n = 50) as follows: control group (sterile water injection); and L-Leu-injected group. The dose of *in ovo* L-Leu and the recording of the hatching performance were as described in Experiment 2. The rearing conditions were the same as described above.

However, sexing was carried out in this experiment on day 2 after hatching. Male broiler chicks were selected for the experiment, because males (Julia strain) had been used in our previous experiments concerning heat-stress administration [5, 6, 17-19]. On day 9, broiler chicks were exposed to HT or CT as described above for layer chicks. All birds were provided the food and water *ad libitum* during the 180 min of the heat challenge. Rectal temperature was recorded at different time points of 0, 60, 120 and 180 min from the start of the heat challenge. To investigate the effect of TM on amino acid metabolism, free amino acid concentrations in the brain and liver were analyzed by HPLC according to the method of Boogers et al. [54], with some modifications as described by Ito et al. [18]. For the amino acid concentrations in Experiment 1, a repeated-measures two-way ANOVA was applied and a *t*-test was used for analysis when a significant interaction was detected. Hatchability, body weight, rectal temperature and time until hatching at DOH were statistically analyzed by one-way ANOVA, and the Tukey-Kramer test was performed as a post-hoc test in Experiment 2. The rectal temperature at hatching was analyzed by *t*-test. Rectal temperature under heat challenge was analyzed by three-way ANOVA in Experiments 3.

Compared with the ST embryos, Leu, Phenylalanine (Phe) and Lysine (Lys) were significantly ($P<0.05$) decreased in the brain and liver in the TM embryos. Significant ($P<0.05$) interactions of TM and time on Leu, Phe, Val and tyrosine (Tyr) in the brain suggested that TM-dependent reduction of these amino acids was pronounced on ED 19, while Leu, but not Phe, also declined on ED 14 and Val and Tyr increased on ED 14. Significant ($P<0.05$) interactions of TM and time on Leu, Phe, Lys and aspartate (Asp) in the liver indicated that the concentrations of the first three free amino acids were significantly depressed at the middle stage of embryonic development (i.e. ED 14); however, Asp was found to have significantly declined at DOH. Although body weight and hatchability were not influenced by the treatments, it was found that rectal temperature was significantly ($P<0.05$) low in the L-Leu treated chicks at DOH. In particular, female chicks were more responsive to declined body temperature at DOH. Although *in ovo* injection of L-Leu did not afford thermotolerance in neonatal (5- and 6 days old) chicks (*data not shown*), L-Leu afforded a significant ($P<0.05$) low rectal temperature in 9 days old male young chicks under HT (Fig. 4). HT significantly ($P<0.001$) increased rectal temperature. The interaction between time and HT had also a significant ($P<0.001$) effect on rectal temperature, suggesting that the effect of HT on increased rectal temperature became more pronounced as time progressed.

TM caused a significant decline in Leu, Phe and Lys in both the embryonic brain and the embryonic liver. In fact, TM induced heat stress in embryos because it increased egg shell temperature and the heart rate of embryos, and stimulated gene expression of heat shock proteins and plasma corticosterone concentration during embryogenesis [36, 55]. Some of the free amino acids (Leu, Phe, Lys and Arg) that were decreased by TM were essential amino acids. It could, therefore, be hypothesized that TM caused the utilization of those amino acids for increased protein synthesis or enhancement of metabolic activities, and thereby decreased free amino acid concentrations in

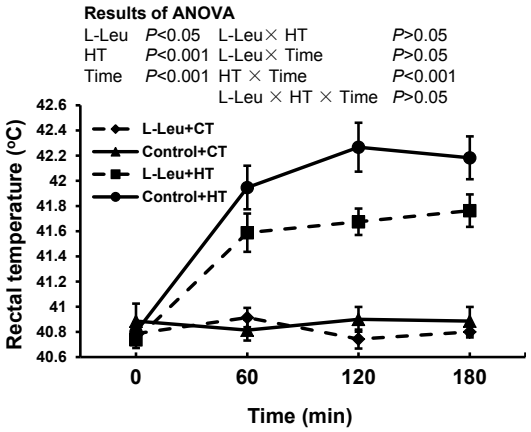

Fig.4. Effects of in ovo L-Leu injection on rectal temperature in young male chicks exposed to either control thermoneutral temperature (CT; 28 ± 1°C) or a high ambient temperature (HT; 35 ± 1°C) for 180 min. The number of chicks in each group was as follows: control (CT = 7; HT= 6); L-Leu-injected groups (CT = 7; HT = 6). Values are mean ± SEM. L-Leu, L-leucine. Reproduced from Han G, Yang H, Bahry MA, Tran PV, Do PH, Ikeda H, Furuse M, Chowdhury VS. 2017. L-Leucine acts as a potential agent in reducing body temperature at hatching and affords thermotolerance in broiler chicks. Comp. Biochem. Physiol. A Mol. Integr. Physiol., 204:48–56, with the permission from Elsevier as the authors' right.

the embryonic brain and liver, because the development of breast muscle and metabolic activities were improved by TM [31, 55]. Among the altered amino acids, Leu was significantly declined in the brain at both ED 14 and ED 19, and it showed a drastic reduction on ED 14 in the liver, which indicates that Leu might be a more critical amino acid to contribute to the embryonic development under TM. One of the highly concentrated amino acids in the egg yolk is Leu (744 μg/g of dry weight) [56], which also suggests an important role of this amino acid in embryonic development. It has been reported that Leu and its metabolites (α-ketoisocaproate (α–KIC) and β-hydroxy-β-methylbutyrate (HMB)) have a robust influence on protein synthesis [57, 58] through the mammalian target of rapamycin signaling pathway [57] and suppression of myofibrillar proteolysis in chick skeletal muscles [59, 60]. Kita et al. [61] further reported that *in ovo* injection of Leu increased embryonic weight at ED 14, but did not affect body weight at hatching. Therefore, Leu, which is an important amino acid in the egg for embryonic development raised some possibilities of having physiological connections with TM.

TM during embryogenesis caused a higher metabolic rate at the beginning of TM; however, rectal temperature was lowered at DOH [31] due to a low metabolic rate before hatching [36, 55]. The mechanism of lowering the rectal temperature by L-Leu injection at hatching might be analogous to the TM mechanism. In Experiment 2, it was shown that L-Leu, but not the combined BCAAs, has the potential function of reducing body temperature at hatching. Although Harper et al. [62] reported that an excessive supplementation of BCAAs may increase the requirement for the other two amino acids, Bak et al. [63] suggested that individual BCAAs have specific effects on the metabolic pathway. Therefore, L-Leu might have significant contribution in metabolic alteration during TM.

In heat challenge study, rectal temperature was significantly increased by HT since commercial broilers become hypothermic under HT [4]. Arad and Itsaki-Glucklish [64] reported that the thermoregulatory mechanism developed in broiler chicks until they were 10 days old. Therefore, it could be hypothesized that 5- or 6-day-old neonatal chicks were not developed enough to show their L-Leu-dependent thermotolerance, but it did appear in 9-day-old young male chicks. As discussed above, if broiler females are more responsive to L-Leu administration than males, it can be assumed that L-Leu injection could also provide more thermotolerance in females than in males. However, our recent study showed that this hypothesis was not correct, i.e. L-Leu *in ovo* injected female broiler chicks did not show thermotolerance (*data not shown*). We are currently analyzing the possible reasons of sex-dependent L-Leu mediated thermotolerance issues, which would clarify the interesting phenomenon of sex-dependent thermoregulation under HT in broilers. Yahav [50] reported that the thermotolerance showed a reduction in heat production in chicks, coupled with increased sensible heat loss, enabled relatively slow development of hyperthermia and thus dramatically reduced mortality. From ED 7, *in ovo*-injected L-Leu, or its metabolites α–KIC and/or HMB, significantly improved the mitochondrial content and mitochondrial biogenesis–related gene expression in myoblast cells [65]. The enhanced mitochondrial density is beneficial to oxidative metabolism and metabolic capacity, which supports the development of regulation in body temperature [50]. Mitochondria are also related with the fatty acid synthesis is organisms. Therefore, the contribution of fatty acids to this process needs to clarify in future. Furthermore, L-Leu could also be further catabolized to acetyl CoA, which participates in the citric acid cycle [66]. The activity of enzymes in the citric acid cycle is also beneficial for improved metabolic capacity and the maintenance of endothermy during embryogenesis [67]. Our recent data (*unpublished*) showed that L-Leu *in ovo* injection stimulated lipid metabolism in embryos, which might cause some metabolic imprinting to trigger some lipid metabolic activities in young chicks under heat challenge for providing more energy under critical period of stress.

4. Concluding remarks

Peripheral L-Cit has a hypothermic function that can afford thermotolerance in neonatal chicks and marketing aged broilers. A further study will focus on elucidating the mechanisms of the peripheral functions of L-Cit in thermoregulation. On the other hand, L-Leu

was found to be a potential candidate for affording thermotolerance in young male chicks. Further research is needed to clarify whether the effect of L-Leu *in ovo* administration on thermotolerance could be persisted until the marketing age of broilers. In conclusion, L-Cit and L-Leu raised the possibility to be considered as novel nutritional therapeutics to face the heat stress challenge in chickens.

Acknowledgements

This study was supported by a Grant-in-Aid for Scientific Research (No. 23248046 to MF and No. 15K07694 to VSC) from the Japan Society for the Promotion of Science and by Kieikai Research Grant Support, 2014 and 2015, to VSC. The diligent technical assistance of Asako Shigemura, Mohammad A. Bahry, Phuong V. Tran and Phong H. Do for L-citrulline chick study are gratefully appreciated. The authors also sincerely acknowledge the members of Laboratory of Animal Behavior and Physiology, Hiroshima University, for their technical support in maintaining the animals for L-citrulline broiler experiment.

References

1) Kjellstrom T, McMichael AJ. 2013. Climate change threats to population health and well-being: the imperative of protective solutions thatwill last. Glob. Health Action, 6:20816.
2) Tanizawa H, Shiraishi J, Kawakami S-I, Tsudzuki M, Bungo T. 2014. Effect of short term thermal conditioning on physiological and behavioral responses to subsequent acute heat exposure in chicks. J. Poult. Sci., 51:80–86.
3) Yahav S, Shinder D, Tanny J, Cohen S. 2005. Sensible heat loss—the broiler's paradox. World's Poult. Sci. J., 61:419–435.
4) Yahav S, Hurwitz S. 1996. Induction of thermotolerance in male broiler chickens by temperature conditioning at an early age. Poult. Sci., 75:402–406.
5) Chowdhury VS, Tomonaga S, Nishimura S, Tabata S, Cockrem JF, Tsutsui K, Furuse M. 2012. Hypothalamic gonadotropin-inhibitory hormone precursor mRNA is increased during depressed food intake in heat-exposed chicks. Comp. Biochem. Physiol. A Mol. Integr. Physiol., 162:227–233.
6) Chowdhury VS, Tomonaga S, Nishimura S, Tabata S, Furuse M. 2012. Physiological and behavioral responses of young chicks to high ambient temperature. J. Poult. Sci., 49:212–218.
7) Bartlett JR, Smith MO. 2003. Effects of different levels of zinc on the performance and immunocompetence of broilers under heat stress. Poult. Sci., 82:1580–1588.
8) Soleimani AF, Kasim A, Alimon AR, Meimandipour A, Zulkifli I. 2010. Ileal endogenous amino acid flow of broiler chickens under high ambient temperature. J. Anim. Physiol. Anim. Nutr. (Berl), 94:641–647.
9) Azad MA, Kikusato M, Maekawa T, Shirakawa H, Toyomizu M. 2010. Metabolic characteristics and oxidative damage to skeletal muscle in broiler chickens exposed to chronic heat stress. Comp. Biochem. Physiol. A Mol. Integr. Physiol., 155:401–406.
10) Ajakaiye JJ, Ayo JO, Ojo SA. 2010. Effects of heat stress on some blood parameters and egg production of Shika Brown layer chickens transported by road. Biol. Res., 43:183–189.
11) Mendes AA, Watkins SE, England JA, Saleh EA, Waldroup AL, Waldroup PW. 1997. Influence of dietary lysine levels and arginine:lysine ratios on performance of broilers exposed to heat or cold stress during the period of three to six weeks of age. Poult. Sci., 76:472–481.
12) Brake J, Balnave D, Dibner JJ. 1998. Optimum dietary arginine : lysine ratio for broiler chickens is altered during heat stress in association with changes in intestinal uptake and dietary sodium chloride. Br. Poult. Sci., 39:639–647.
13) Willemsen H, Swennen Q, Everaert N, Geraert PA, Mercier Y, Stinckens A, Decuypere E, Buyse J. 2011. Effects of dietary supplementation of methionine and its hydroxy analog DL-2-hydroxy-4-methylthiobutanoic acid on growth performance, plasma hormone levels, and the redox status of broiler chickens exposed to high temperatures. Poult. Sci., 90:2311–2320.

14）Dai SF, Gao F, Xu XL, Zhang WH, Song SX, Zhou GH. 2012. Effects of dietary glutamine and gamma-aminobutyric acid on meat colour, pH, composition, and water-holding characteristic in broilers under cyclic heat stress. Br. Poult. Sci., 53:471–481.
15）Erwan E, Tomonaga S, Yoshida J, Nagasawa M, Ogino Y, Denbow DM, Furuse M. 2012. Central administration of L- and D-aspartate attenuates stress behaviors by social isolation and CRF in neonatal chicks. Amino Acids, 43:1969–1976.
16）Erwan E, Chowdhury VS, Nagasawa M, Goda R, Otsuka T, Yasuo S, Furuse M. 2014. Central injection of L- and D-aspartate attenuates isolation-induced stress behavior in chicks possibly through different mechanisms. Eur. J. Pharmacol., 736:138–142.
17）Chowdhury VS, Tomonaga S, Ikegami T, Erwan E, Ito K, Cockrem JF, Furuse M. 2014. Oxidative damage and brain concentrations of free amino acid in chicks exposed to high ambient temperature. Comp. Biochem. Physiol. A Mol. Integr. Physiol., 169:70–76.
18）Ito K, Erwan E, Nagasawa M, Furuse M, Chowdhury VS. 2014. Changes in free amino acid concentrations in the blood, brain and muscle of heat-exposed chicks. Br. Poult. Sci., 55:644–652.
19）Ito K, Bahry MA, Hui Y, Furuse M, Chowdhury VS. 2015. Acute heat stress upregulates neuropeptide Y precursor mRNA expression and alters brain and plasma concentrations of free amino acids in chicks. Comp. Biochem. Physiol. A Mol. Integr. Physiol., 187:13–19.
20）Koga Y, Ohtake R. 1914. Study report on the constituents of squeezed watermelon. J. Tokyo Chem. Soc., 35:519–528.
21）Wada M. 1930. On the occurrence of a new amino acid in watermelon. Proc. Imp. Acad., 6:15–17.
22）Rimando AM, Perkins-Veazie PM. 2005. Determination of citrulline in watermelon rind. J. Chromatogr. A., 1078:196–200.
23）Tarazona-Díaz MP, Viegas J, Moldao-Martins M, Aguayo E. 2011. Bioactive compounds from flesh and by-product of fresh-cut watermelon cultivars. J. Sci. Food Agric., 91:805–812.
24）Curis E, Nicolis I, Moinard C, Osowska S, Zerrouk N, Bénazeth S, Cynober L. 2005. Almost all about citrulline in mammals. Amino Acids, 29:177–205.
25）Schwedhelm E, Maas R, Freese R, Jung D, Lukacs Z, Jambrecina A, Spickler W, Schulze F, Böger RH. 2008. Pharmacokinetic and pharmacodynamic properties of oral L-citrulline and L-arginine: impact on nitric oxide metabolism. Br. J. Clin. Pharmacol., 65:51–59.
26）De Luca B, Monda M, Sullo A. 1995. Changes in eating behavior and thermogenic activity following inhibition of nitric oxide formation. Am. J. Physiol., 268:R1533–R1538.
27）Gourine AV. 1995. Pharmacological evidence that nitric oxide can act as an endogenous antipyretic factor in endotoxin-induced fever in rabbit. Gen. Pharmacol., 26:835–841.
28）Yahav S, Collin A, Shinder D, Picard M. 2004. Thermal manipulations during broiler chick embryogenesis: effects of timing and temperature. Poult. Sci., 83:1959–1963.
29）Collin A, Picard M, Yahav S. 2005. The effect of duration of thermal manipulation during broiler chick embryogenesis on body weight and body temperature of post-hatched chicks. Anim. Res., 54:105–111.
30）Piestun Y, Shinder D, Ruzal M, Halevy O, Brake J, Yahav S. 2008. Thermal manipulations during broiler embryogenesis: effect on the acquisition of thermotolerance. Poult. Sci., 87:1516–1525.
31）Loyau T, Métayer-Coustard S, Berri C, Crochet S, Cailleau-Audouin E, Sannier M, Chartrin P, Praud C, Hennequet-Antier C, Rideau N, Courousse N, Mignon-Grasteau S, Everaert N, Duchlos MJ, Yahav S, Tesseraud S, Collin A. 2014. Thermal manipulation during embryogenesis has long-term effects on muscle and liver metabolism in fast-growing chickens. PLoS ONE, 9:e105339.
32）Piestun Y, Druyan S, Brake J, Yahav S. 2013. Thermal manipulations during broiler incubation alter performance of broilers to 70 days of age. Poult. Sci., 92:1155–1163.

33）Yalçın S, Çabuk M, Bruggeman V, Babacanoğlu E, Buyse J, Decuypere E, Siegel PB. 2008. Acclimation to heat during incubation: 3. Body weight, cloacal temperatures, and blood acid-base balance in broilers exposed to daily high temperatures. Poult. Sci., 87:2671–2677.]

34）Al-Zhgoul MB, Dalab AE, Ababneh MM, Jawasreh KI, Al-Busadah KA, Ismail ZB. 2013. Thermal manipulation during chicken embryogenesis results in enhanced Hsp70 gene expression and the acquisition of thermotolerance. Res. Vet. Sci., 95:502–507.

35）Walstra I, Ten Napel J, Kemp B, Van den Brand H. 2010. Temperature manipulation during layer chick embryogenesis. Poult. Sci., 89:1502–1508.

36）Piestun Y, Halevy O, Yahav S. 2009. Thermal manipulations of broiler embryos-the effect on thermoregulation and development during embryogenesis. Poult. Sci., 88:2677–2688.

37）Han G, Yang H, Bahry MA, Tran PV, Do PH, Ikeda H, Furuse M, Chowdhury VS. 2017. L-Leucine acts as a potential agent in reducing body temperature at hatching and affords thermotolerance in broiler chicks. Comp. Biochem. Physiol. A Mol. Integr. Physiol., 204:48–56.

38）Davis JL, Masuoka DT, Gerbrandt LK, Cherkin A. 1979. Autoradiographic distribution of L-proline in chicks after intracerebral injection. Physiol. Behav., 22:693–695.

39）Furuse M, Ando R, Bungo T, Ao R, Shimojo M, Masuda Y. 1999. Intracerebroventricular injection of orexins does not stimulate food intake in neonatal chicks. Br. Poult. Sci., 40:698–700.

40）Koutoku T, Takahashi H, Tomonaga S, Oikawa D, Saito S, Tachibana T, Han L, Hayamizu K, Denbow DM, Furuse M. 2005. Central administration of phosphatidylserine attenuates isolation stress-induced behavior in chicks. Neurochem. Int., 47:183–189.

41）Windmueller HG, Spaeth AE. 1981. Source and fate of circulating citrulline. Am. J. Physiol., 241:473–480.

42）Tamir H, Ratner S. 1963. Enzymes of arginine metabolism in chicks. Arc. Biochem. Biophys., 102:249–258.

43）Suenaga R, Yamane H, Tomonaga S, Asechi M, Adachi N, Tsuneyoshi Y, Kurauchi I, Sato H, Denbow DM, Furuse M. 2008. Central L-arginine reduced stress responses are mediated by L-ornithine in neonatal chicks. Amino Acids, 35:107–113.

44）Alberts B, Bray D, Hopkins K, Johnson A, Lewis J, Raff M, Roberts K, Walter P. 2014. Energy generation in mitochondria and chloroplast. In: Essential Cell Biology. 4th ed. Garland Science, Taylor and Francis Group Press. USA.

45）Palmer RMJ, Ferrige AG, Moncada S. 1987. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature, 327:524–526.

46）Szabo C. 1996. Physiological and pathophysiological roles of nitric oxide in the central nervous system. Brain Res. Bull., 41:131–141.

47）Chowdhury VS, Shigemura A, Erwan E, Ito K, Bahry MA, Tran PV, Furuse M. 2015. Oral administration of L-citrulline, but not L-arginine or L-ornithine, acts as a hypothermic agent in chicks. J. Poult. Sci., 52:331–335.

48）Fang YZ, Yang S, Wu G. 2002. Free radicals, antioxidants, and nutrition. Nutrition, 10:872–879.

49）Yalçın S, Bruggeman V, Buyse J, Decuypere E, Cabuk M, Siegel PB. 2009. Acclimation to heat during incubation: 4. Blood hormones and metabolites in broilers exposed to daily high temperatures. Poult. Sci., 88:2006–2013.

50）Yahav S. 2015. Regulation of body temperature: strategies and mechanisms. Sturkie's Avian Physiology, 6th ed. Academic Press, Elsevier, CA, USA.

51）Gan JC, Jeffay H. 1967. Origins and metabolism of the intracellular amino acid pools in rate liver and muscle. Biochim. Biophys. Acta., 148:448–459.

52）Ohta Y, Tsushima N, Koide K, Kidd M, Ishibashi T. 1999. Effect of amino acid injection in broiler breeder eggs on embryonic growth and hatchability of chicks. Poult. Sci., 78:1493–1498.

53）Ohta Y, Kidd MT, Ishibashi T. 2001. Embryo

growth and amino acid concentration profiles of broiler breeder eggs, embryos, and chicks after in ovo administration of amino acids. Poult. Sci., 80:1430–1436.

54）Boogers I, Plugge W, Stokkermans YQ, Duchateau AL. 2008. Ultra-performance liquid chromatographic analysis of amino acids in protein hydrolysates using an automated pre-column derivatisation method. J. Chromatogr. A., 1189:406–409.

55）Piestun Y, Yahav S, Halevy O. 2015. Thermal manipulation during embryogenesis affects myoblast proliferation and skeletal muscle growth in meat-type chickens. Poult. Sci., 94:2528–2536.

56）Nimalaratne C, Lopes-Lutz D, Schieber A, Wu J. 2011. Free aromatic amino acids in egg yolk show antioxidant properties. Food Chem., 129:155–161.

57）Deng H, Zheng A, Liu G, Chang W, Zhang S, Cai H. 2014. Activation of mammalian target of rapamycin signaling in skeletal muscle of neonatal chicks: effects of dietary leucine and age. Poult. Sci., 93:114–121.

58）Duan Y, Li F, Li Y, Tang Y, Kong X, Feng Z, Anthony TG, Watfoud M, Hou Y, Wu G, Yin Y. 2016. The role of leucine and its metabolites in protein and energy metabolism. Amino Acids, 48:41–51.

59）Nakashima K, Ishida A, Yamazaki M, Abe H. 2005. Leucine suppresses myofibrillar proteolysis by down-regulating ubiquitin-proteasome pathway in chick skeletal muscles. Biochem. Biophys. Res. Commun., 336:660–666.

60）Nakashima K, Yakabe Y, Ishida A, Yamazaki M, Abe H. 2007. Suppression of myofibrillar proteolysis in chick skeletal muscles by α-ketoisocaproate. Amino Acids, 33:499–503.

61）Kita K, Ito KR, Sugahara M, Kobayashi M, Makino R, Takahashi N, Nakahara H, Takahashi K, Nishimukai M. 2015. Effect of in ovo administration of branched chain amino acids on embryo growth and hatching time of chickens. J. Poult. Sci., 52:34–36.

62）Harper AE, Benevenga NJ, Wohlhueter RM. 1970. Effects of ingestion of disproportionate amounts of amino acids. Physiol. Rev., 50:428–558.

63）Bak LK, Johansen ML, Schousboe A, Waagepetersen HS. 2012. Valine but not leucine or isoleucine supports neurotransmitter glutamate synthesis during synaptic activity in cultured cerebellar neurons. J. Neurosci. Res., 90:1768–1775.

64）Arad Z, Itsaki-Glucklish S. 1991. Ontogeny of brain temperature in quail chicks (Coturnix coturnix japonica). Physiol. Zool., 64:1356–1370.

65）Liang C, Curry BJ, Brown PL, Zemel MB. 2014. Leucine modulates mitochondrial biogenesis and SIRT1-AMPK signaling in C2C12 myotubes. J. Nutr. Metab., 2014:239750.

66）Shimomura Y, Kitaura Y, Kadota Y, Ishikawa T, Kondo Y, Xu M, Ota M, Morishita Y, Bariuan JV, Zhen H. 2015. Novel physiological functions of branched chain amino acids. J. Nutr. Sci. Vitaminol. (Tokyo), 61:S112–S114.

67）Walter I, Seebacher F. 2009. Endothermy in birds: underlying molecular mechanisms. J. Exp. Biol., 212:2328–2336.

Bean husk, an agricultural by-product having functionality in animal nutrition and health

Htun Myint, Hiroyuki Kishi, Yu Iwahashi, Yutaka Suzuki, Satoshi Koike and Yasuo Kobayashi*

（*Graduate School of Agriculture, Hokkaido University, Kita 9 Nishi 9, Sapporo 060-8589, Japan*）

Abstract

Bean husk is an agricultural by-product abundantly available in the world and considered a new feed source candidate for various animals. Although bean husk has been reported as a fiber supplement for ruminant animals, evaluation of its functional property with respect to nutrition and health in monogastric animals is quite limited. A series of studies using chickpea (*Cicer arietinum*) husk, lablab bean (*Dolichos lablab*) husk and soybean (*Glycine max*) husk were conducted to evaluate the functional properties of the husk in monogastric animals. First, we evaluated dietary supplementation (5%) of chickpea husk using rats and found that rats fed chickpea husk showed lower levels of cholesterol and thiobarbituric acid reactive substances (TBARS) in blood by its polyphenolic component. Second, we evaluated lablab bean husk or soybean husk supplemented (5%) diet using rats and found that rats fed either husk showed increased cecal short chain fatty acid (SCFA) than those on cellulose (control) and starch (negative control). Lablab bean husk increased cecal abundance of *Akkermansia,* while soybean husk increased that of lactobacilli according to qPCR and Miseq analyses. Both types of bean husk were found to contain oligosaccharides and these might selectively stimulate the growth of beneficial bacteria and improve hindgut fermentation. Cellulose- or soybean husk-supplemented (5.6%) feed was given to Shiba dogs. Dogs fed soybean husk showed higher SCFA level and higher abundance of lactobacilli and bacterial groups including butyrate producers in their feces than those fed cellulose. Based on these, three different types of bean husk tested in the present study were suggested as a functional feed ingredient for possibly promoting blood or gut health of monogastric animals.

Key words: antioxidants, bean husk, cholesterol, hindgut fermentation, microbes

1. Introduction

Bean husk is an agricultural by-product yielded during the separation of bean and has attracted attention to efficient utilization. Chickpea husk constitutes about 10-11% of the whole seed and the presence of tannin and dietary fiber has been reported[1, 2]. Lablab bean is produced mainly in South and South East Asian countries and its husk (8% of bean) is suggested to be used as easily digestible supplemental fiber that selectively stimulates rumen fibrolytic bacteria in ruminant animals[3]. Fiber components of lablab bean husk are represented by cellulose, hemicellulose and pectic polysaccharide including arabinogalactan[4]. Although the presence of oligosaccharides in lablab bean husk has been indicated formerly, those sugars are not characterized in detail. Soybean husk constitutes 8-10% of seed, contains a rapidly fermentable fiber, and widely used as a palatable energy source for ruminants but without assessing its functionality. Fiber components of soybean husk are cellulose, hemicellulose, pectin, uronic acid and lignin[5], some of which can be considered a functional dietary fiber.

Therefore, various types of bean husk could be a

Proceedings of Japanese Society for Animal Nutrition and Metabolism 61 (2): 13-19, 2017.

*Correspondence to:
Yasuo Kobayashi, PhD
Email: kyas@anim.agr.hokudai.ac.jp

functional feed ingredient for monogastric animals. As a supplement of functional components such as polyphenols or dietary fiber, bean husk in the diet of monogastric animal is expected to improve health status of the animal through antioxidant activity and hindgut microbial modulation. However, studies on bean husk for monogastric animal are still limited. Therefore, we describe here about potential evaluation of different types of bean husk in a series of feeding studies using rats and dogs, which may contribute to the understanding of new functional feed candidates for the enhancement of health of monogastric animals.

2. Functional components of bean husks

In our studies, chickpea husk, lablab bean husk and soybean husk were found to contain both of soluble (represented by non-structural carbohydrate) and insoluble (neutral detergent fiber) dietary fiber. However, each bean husk contains different amounts of polyphenol and fiber (determined by AOAC 1990; Goering & Van Soest 1975; Makkar *et al*. 1993) [6-8]. In particular, chickpea husk was rich in tannin, resulting in high antioxidative effects (Table 1) as previously reported [9]. Meanwhile, soluble sugar from lablab bean husk and soybean husk appeared as two main spots on thin layer chromatography, and were identified as raffinose and stachyose (Fig 1) . When these different types of bean husk are used in nutritional intervention studies, different functional properties can be expected. We have carried out three feeding studies using rat and dogs. Main findings are described as follows.

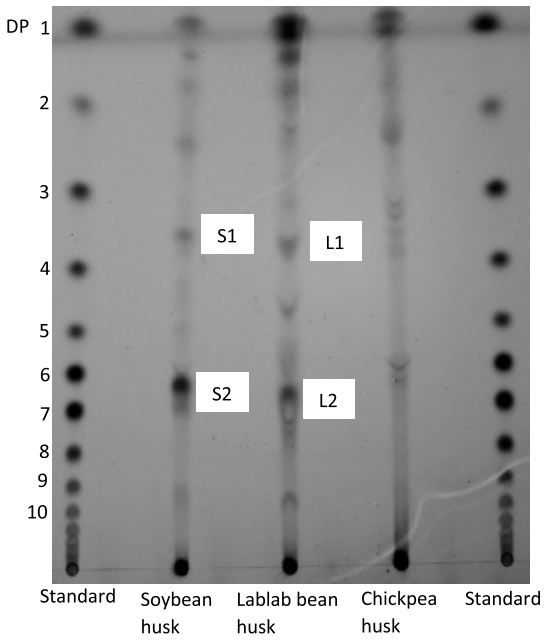

Figure 1. Thin layer chromatography to detect oligosaccharides. L1, L2, S1, and S2 are prominent oligosaccharide fractions that have been identified raffinose (S1 and L1) and stachyose (S2 and L2). DP, degree of polymerization.

3. Function of chickpea husk

Functional property of chickpea husk was evaluated

Table 1. Chemical composition of three different types of bean husk

Parameters	Soybean husk	Lablab bean husk	Chickpea husk†
	---------- dry matter basis ----------		
Neutral detergent fiber %	61.0	66.6	61.6
Acid detergent fiber %	47.0	52.2	58.6
Acid detergent lignin %	0.2	0.2	3.0
Non-structural carbohydrate %	24.0	23.6	28.5
Soluble fiber %	6.0	2.0	3.0
Crude ash %	4.5	3.3	4.0
Crude protein %	9.2	5.9	4.5
Ether extract %	1.4	1.2	1.0
Total tannin %	0.2	0.4	2.1
Superoxide dismutase %	20.1	23.5	32.0
DPPH %	1.0	1.0	31.0

Non-structural carbohydrate was calculated as 100-(NDF+ash+protein+fat), Soluble fiber was analyzed by Dietary Fiber Assay Kits (K-TDFR, Megazyme, Ireland), † Data are referred to Myint *et al.* 2017a [24]

by using 15 Sprague-Dawley rats at 5 wks of age. They were grouped into three treatments (5 rats/group) with average body weight basis. All rats had free access to water and diet. One group was given a purified diet (AIN-93G, CLEA Japan, Inc., Tokyo. Japan) containing 5% cellulose powder used as control diet. The other two groups were given the same purified diets in which 5% cellulose was replaced by either 5% corn starch, or 5% chickpea husk. Starch diet did not contain any fiber source and was regarded as a negative control. Chickpea husk (Desi type) was imported from Myanmar. After 3 weeks feeding, rats were anaesthetized with injection of xylazine·HCl. Blood samples were taken from the heart, and separated blood plasma was analyzed for cholesterol (T-Cho CII kits, Wako, Osaka, Japan) and thiobarbituric acid reactive substances (TBARS) by assay kit (Cayman chemical, Michigan, USA).

The lower concentration of total cholesterol concentration ($P < 0.05$) was observed in rats fed chickpea husk diet in comparison with starch (Fig. 2A). Lower cholesterol level is probably due to tannins and dietary fiber present in chickpea husk. Tannins of chickpea husk can bind to bile acid in rat intestine and inhibit its reabsorption, facilitating bile acid synthesis in the liver for which plasma cholesterol is used. This can promote the reduction of blood cholesterol level. Beside tannins, both of soluble and insoluble fiber in chickpea husk might in part inhibit absorption of cholesterol source from dietary lipids by acceleration of fecal lipid excretion through lipid binding ability of tannins.

Blood lipid oxidation was measured by using TBARS and chickpea husk dietary supplementation showed the reduction of TBARS ($P < 0.05$) in comparison with cellulose and starch supplementation (Fig. 2B) . Tannins in chickpea husk might be responsible for the prevention of superoxide formation and lipid peroxidation. In fact, TBARS lowering effect of tannin has been demonstrated in serum of rabbits [10]. Tannin-rich fiber found in tea was reported as a lipid binding agent that reduced blood cholesterol level and improved antioxidant activity in mice, because tannins interfere with fatty acid metabolism and it enhance endogenous antioxidant defense system or participate in the regeneration of other antioxidant

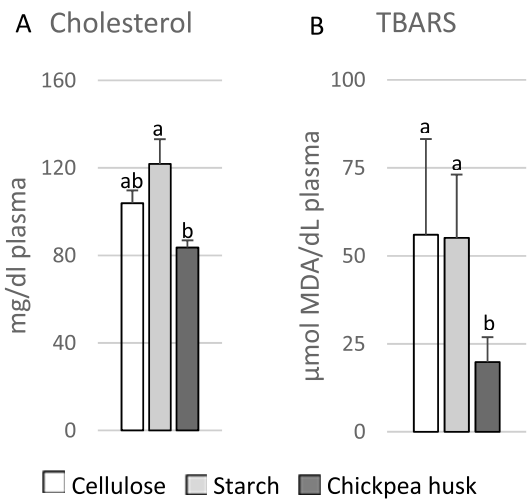

Figure 2. Blood cholesterol (A) and thiobarbituric acid reactive substances (TBARS) (B) of rats fed three different diets: Cellulose (Control), AIN 93G diet containing 5% cellulose, Starch (Negative control), AIN 93G diet in which cellulose is replaced by corn starch and Chickpea husk (Treatment) AIN 93G diet in which cellulose is replaced by chickpea husk. a, b denote $P < 0.05$. Data are referred to Myint *et al*. 2017a [24].

compounds [11]. Cholesterol-lowering effect of chickpea husk and protective effect of chickpea husk against lipid oxidation suggest the functional importance of this by-product in prevention and care for cardiovascular diseases and overall health in animals.

4. Function of lablab bean husk

Twenty male Sprague-Dawley rats aged 5 wks were divided into four groups (n=5). Diet and water were provided *ad libitum*. One group was given a purified diet that contains 5% cellulose powder as fiber source. This was used as the control diet. The other three groups were given the same purified diet in which 5% cellulose was replaced by either corn starch, lablab bean husk, or soybean husk. Starch diet was regarded as a negative control. Lablab bean husk generated from local pulse processing plant (Yezin, Nay Pyi Taw, Myanmar) where bean were soaked and de-hulled by hand. Soybean husk (Japanese product) was obtained through ZEN-NOH (Tokyo). After 3 weeks' feeding, rats were sacrificed to take cecal digesta for the analysis of SCFA by gas chromatography as described by Suto (1973) [12] and microbiota (by qPCR and Miseq according to Yu & Morrison 2004; Caporaso et al. 2010;

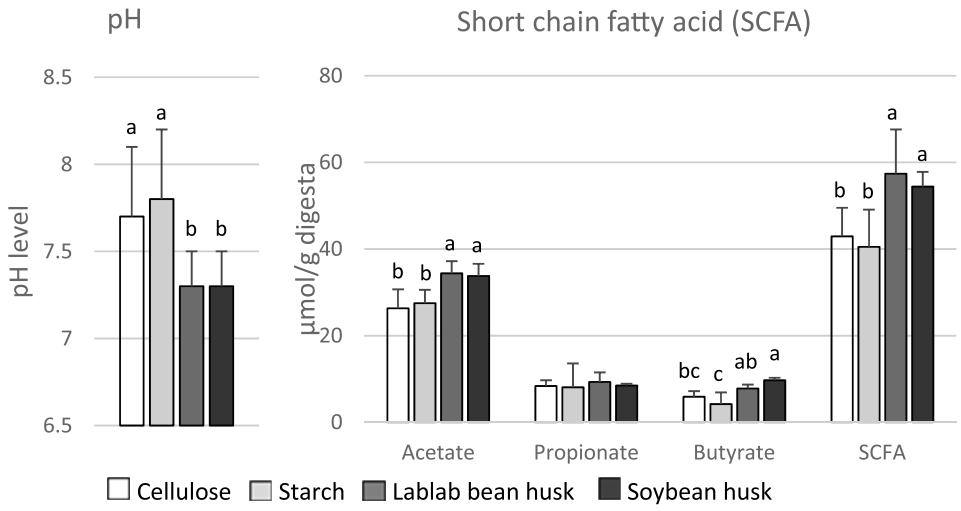

Figure 3. Cecal SCFA and pH of rats fed 4 different diets: Cellulose (Control), AIN 93G diet containing 5% cellulose, Starch (Negative control), AIN 93G diet in which cellulose is replaced by corn starch, Lablab bean husk (Treatment 1) AIN 93G diet in which cellulose is replaced by lablab bean husk and Soybean husk (Treatment 2), AIN 93G diet in which cellulose is replaced by soybean husk. a, b, c denote $P < 0.05$.

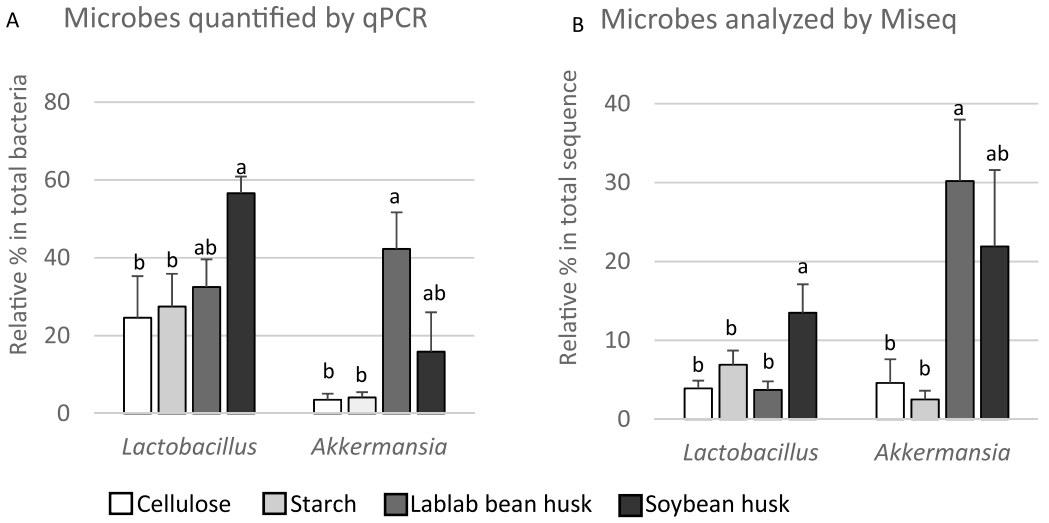

Figure 4. Cecal microbial changes quantified by real-time PCR (qPCR) (A) and analyzed by Miseq (B) of rats fed 4 different diets: Cellulose (Control), AIN 93G diet containing 5% cellulose, Starch (Negative control), AIN 93G diet in which cellulose is replaced by corn starch, Lablab bean husk (Treatment 1) AIN 93G diet in which cellulose is replaced by lablab bean husk and Soybean husk (Treatment 2), AIN 93G diet in which cellulose is replaced by soybean husk. a, b denote $P < 0.05$.

Herleman et al. 2011) [13-15].

Lablab bean husk feeding increased cecal level of total SCFA ($P < 0.05$) including acetate and butyrate but lowered cecal pH (Fig. 3) , indicating improvement of hindgut fermentation. In particular, lablab bean husk feeding showed higher acetate level that was related to increased abundance of *Akkermansia* ($P < 0.05$) confirmed by both of qPCR and Miseq (Fig 4) . This mucin degrading species is known to convert mucins to acetate [16].

Such prebiotics-like activity might be due to pectin or oligosaccharides possibly contained in lablab bean husk. Consumption of pectin or guar gum was found to increase *Akkermansia* in rats fed high-fat diets [17] and its abundance has been negatively associated with metabolic diseases such as obesity and diabetes [18, 19]. Therefore, increased abundance of *Akkermansia* by lablab bean husk feeding has a beneficial health impact with respect to prevention and care for obesity, diabetes and their associated metabolic

disorders in monogastric animals, especially long-living animals including companion animals and even humans.

5. Function of soybean husk

Soybean husk feeding in rats increased total SCFA ($P < 0.05$), acetate, butyrate in comparison with cellulose and starch feeding (Fig 3). Major polysaccharides and oligosaccharides in soybean husk seem to be utilized by cecal microbes and improved the cecal environment through organic acid production, leading to low pH. The most favorable aspect of soybean husk feeding in rats was the increase of cecal lactobacilli ($P < 0.05$) which were predominant acetate and lactate producers confirmed by both qPCR and Miseq (Fig. 4). The presence of pectin or raffinose family oligosaccharides in soybean husk might be the main factor to increase lactobacilli, because lactobacilli have the ability to break down raffinose and stachyose by their α-galactosidase activity [20], leading greater SCFA production. Soybean husk could possess a beneficial health impact in another aspect, since importance of lactobacilli have been known for the immune development in the gastrointestinal tract [21].

Soybean husk was supplemented in the diet of dogs to evaluate its impact. Four Shiba dogs (7-48 months in age and 7.5±1.7 kg in body weight) were offered twice daily commercial diet (170g/d, Unicharm or Hill's Colgate, Tokyo, Japan) to which 10 g cellulose powder or soybean husk powder was supplemented (5.6% inclusion in total diet). Dogs were fed cellulose-supplemented diet for the first 7 days and then switched to soybean husk-supplemented diet for another 7 days. On 6th and 7th day of each dietary period, fecal collection was made four times. Fecal sample was suspended in distilled water, vortexed, and centrifuged and the supernatant was used for the analysis of fermentation metabolites. Fecal sample was also employed for DNA extraction to monitor fecal microbes by qPCR.

Feces of dogs fed on soybean husk diet had higher levels of total SCFA ($P < 0.05$) and lactate ($P < 0.01$) than those on cellulose diet (Table 2) . These increases of beneficial organic acids with concomitant reduction of pH are indicative of increased microbial growth and activities. Lactogenic effect ($P < 0.05$) of soybean husk found in rat study was confirmed in dog. This caused increase of lactate that might be consumed by clostridial cluster IV including *Faecalibacterium prausnitzii* and clostridial cluster XIVa to produce butyrate as demonstrated by Duncan *et al*. (2004) [22]. Increased abundance of those butyrate producers ($P < 0.05$) are considered favorable, because these species have been shown to possess anti-inflammatory properties due to their potential protective effect on inflammatory bowel disease in dog by producing butyrate [23]. Therefore, supplementation of soybean husk in the diet of dog has a beneficial health impact with respect to its lactogenic effect and potential anti-inflammatory properties.

Table 2. Fecal fermentation metabolites and microbiota of dogs fed experimental diets (Mean ± SD)

Parameters	Diet supplemented with		P-value
	Cellulose[1]	Soybean husk[2]	
pH	6.4 ± 0.6	5.9 ± 0.3	0.004
Metabolites			
Total short chain fatty acid (μmol/g feces)	139.1 ± 28.4	158.3 ± 25.3	0.035
Lactate (μmol/g feces)	13.9 ± 4.5	21 ± 7.4	0.007
Microbiota (Relative % in total bacteria)			
Total lactobacilli	5.6 ± 2.7	14.2 ± 4.5	0.044
Clostridial cluster IV	1.2 ± 0.9	2.8 ± 0.6	0.034
Faecalibacterium prausnitzii	0.4 ± 0.3	2.3 ± 1.1	0.044
Clostridial cluster XIVa	18.4 ± 5.8	33.3 ± 9.7	0.043

[1] Cellulose: 170 g basal feed supplemented with 10 g cellulose was fed daily.
[2] Soybean husk: 170 g of basal feed supplemented with 10 g soybean husk was fed daily.
Data are referred to Myint *et al*. 2017b [25].

6. Conclusion

The results in first experiment indicate that chickpea husk dietary supplementation (5%) improves cardiovascular health through lowering of blood cholesterol and lipid peroxidation product (TBARS) by its polyphenols and dietary fibers in chickpea husk. The results in second experiment indicate that 5 % inclusion of lablab bean husk and soybean husk in the diet promotes cecal fermentation of rats, as represented by higher SCFA. Lablab bean husks exhibits a remarkable selectivity against *Akkermansia,* while soybean husk particularly favors the growth of lactobacilli in the cecum of rats. Improvement of hindgut fermentation and lactogenic effect by soybean husk were confirmed in third experiment using dogs. The improvement of hindgut fermentation and increased proportion of beneficial bacteria by supplementation of bean husk may be useful for promoting gastrointestinal health of monogastric animals. However, further studies are needed to fully understand the association of bean husk feeding with health of animals including humans.

References

1) Sreerangaraju G, Krishnamoorthy U, Kailas MM. 2000. Evaluation of Bengal gram (*Cicer arietinum*) husk as a source of tannin and its interference in rumen and post-rumen nutrient digestion in sheep. Anim. Feed Sci. Technol., 85: 131-138.

2) Ngwe T, Nukui Y, Oyaizu S, Takamoto G, Koike S, Ueda K, Nakatsuji H, Kondo S, Kobayashi Y. 2012. Bean husks as a supplemental fiber for ruminants: potential use for activation of fibrolytic rumen bacteria to improve main forage digestion. Anim. Sci. J., 83: 43-49.

3) Fuma R, Oyaizu S, Nukui Y, Ngwe T, Shinkai T, Koike S, Kobayashi Y. 2012. Use of bean husk as an easily digestible fiber source for activating the fibrolytic rumen bacterium *Fibrobacter succinogenes* and rice straw digestion. Anim. Sci. J., 83: 696-703.

4) Muralikrishna G, Tharanathan RN. 1994. Characterization of pectic polysaccharides from pulse husks. Food Chem., 50: 87-89.

5) Mullin WJ, Xu W. 2001. Study of soybean seed coat components and their relationship to water absorption. J. Agri. Food Chem., 49*:* 5331-5335.

6) AOAC. 1990. Official Methods of Analysis, 15th edn. Association of Official Analytical Chemistry, Virginia.

7) Goering HK, Van Soest PJ. 1975. Forage Fiber Analysis. Agricultural Research Service. Agricultural handbook No. 379. US Department of Agriculture, Washington.

8) Makkar HPS, Blummel M, Borowy NK, Becker K. 1993. Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. J. Sci. Food Agri., 61*:* 161-165.

9) Brand-Williams W, Cuvelier ME, Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft and Technologie, 28*:* 25-30.

10) Jurgonski A, Juskiewicz J, Zdunczyk Z, Matusevicius P, Kolodziejczyk K. 2014. Polyphenol-rich extract from blackcurrant pomace attenuates the intestinal tract and serum lipid changes induced by a high-fat diet in rabbits. Euro. J. Nutr., 53: 1603-1613.

11) Guo W, Shu Y, Yang X. 2016. Tea dietary fiber improves serum and hepatic lipid profiles in mice fed a high cholesterol diet. Plant Foods Human Nutr., 71: 145-150.

12) Suto T. 1973. Examination of the rumen, *In:* Nakamura R, Yonemura T, Suto T *(eds),* Methods in the clinical examination of the bovine, *pp.* 39–42. Nosan Gyoson Bunka Kyokai, Tokyo.

13) Yu Z, Morrison M. 2004. Improved extraction of PCR-quality community DNA from digesta and fecal samples. BioTechniques, 36*:* 808-812.

14) Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley GA, Kelly ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Servinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods, 7: 335-336.

15) Herlemann DP, Labrenz M, Jurgens K, Bertilsson S, Waniek JJ, Andersson AF. 2011. Transitions in

bacterial communities along the 2000 km salinity gradient of the Baltic Sea. ISME J., 5: 1571-1579.

16) Dao MC, Everard A, Aron-Wisnewsky J, Sokolovska N, Prifti E, Verger EO, kayser BD, Levenez F, Chilloux J, Hoyles L, Consortium MO, Dumas ME, Rizkalla SW, Dore J, Cani PD, Clement K. 2016. *Akkermansia muciniphila* and improved metabolic health during a dietary intervention in obesity: relationship with gut microbiome richness and ecology. Gut, 65: 429-436.

17) Jakobsdottir G, Xu J, Molin g, Ahrne S, Byman M. 2013. High-fat diet reduces the formation of butyrate, but increases succinate, inflammation, liver fat and cholesterol in rats, while dietary fibre counteracts these effects. PLoS One, 8: e80476.

18) Everard A, Lazarevic V, Derrien M, Girard M, Muccioli GG, Neyrinck AM, Possemiers S, Van Holle A, Francois P, de Vos WM, Delzenne NM, Schrenzel J, Cani PD. 2011. Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice. Diabetes, 60: 2775-2786.

19) Santacruz A, Collado MC, Garcia-Valdes L, Segura MT, Martin-Lagos JA, Anjos T, Marti-Romero M, Lopez RM, Florido J, campoy C, Sanz Y. 2010. Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. Br. J. Nutr., 104: 83-92.

20) Pan Q, Zeng X, Pan D, Peng L. Wu Z, Sun Y, Wei Y. 2017. The proliferation mechanism of *Lactobacillus plantarum* RBi stimulated by stachyose. Current Microbiol., 74: 732-738.

21) Van Baarlen P, Wells JM, Kleerebezem M. 2013. Regulation of intestinal homeostasis and immunity with probiotic lactobacilli. Trends Immunol., 34: 208-215.

22) Duncan SH, Louis P, Flint HJ. 2004. Lactate-utilizing bacteria, isolated from human feces that produce butyrate as a major fermentation product. Appl. Environ. Microbiol., 70: 5810-5817.

23) Suchodolski JS, Markel ME, Garcia-Mazcorro JF, Unterer S, Heilmann RM, Dowd SE, Kachroo P, Ivanov I, Minamoto Y, Dillman EM, Steiner JM, CookAK, Toresson L. 2012. The fecal microbiome in dogs with acute diarrhea and idiopathic inflammatory bowel disease. PLoS ONE, 7: e51907.

24) Myint H, Kishi H, Koike S, Kobayashi Y. 2017a. Effect of chickpea husk dietary supplementation on hindgut fermentation and microbiota in rats. Anim. Sci. J., 88: 372-378.

25) Myint H, Iwahashi Y, Koike S, Kobayashi Y. 2017b. Effect of soybean husk supplementation on the fecal fermentation metabolites and microbiota of dogs. Anim. Sci. J., doi:10.1111/asj.12817.

日本および東南アジアにおけるウシからの消化管発酵由来メタン排出抑制の可能性

鈴木知之[1]・寺田文典[2]・川島知之[3]
（国際農林水産業研究センター[1]、東北大学大学院農学研究科[2]、宮崎大学農学部[3]）

1. はじめに

大気中の二酸化炭素、メタン等の温室効果ガスの濃度上昇とそれに伴う地球規模の気候変動緩和のため、京都議定書に代わる、温室効果ガス削減等のための新たな国際枠組みであるパリ協定が2016年11月に発効された。本協定は、産業革命前からの地球平均気温の上昇を2° Cより十分下方に保持するとともに、気温上昇を1.5° Cに抑える努力を追及することを長期目標としている。これに先立ち、日本政府は2030年度の温室効果ガス削減目標を2013年度比26％減とする約束草案を国連気候変動枠組条約事務局に提出した。

畜産に起因する主要な温室効果ガスとしては、反すう家畜の消化管内で生産されるメタン、および家畜排せつ物の堆積や処理時に発生するメタンおよび一酸化二窒素があげられ、メタンおよび一酸化二窒素の温室効果能はそれぞれ、二酸化炭素の25倍および298倍とされている[1)]。家畜生産に伴う温室効果ガスの年間排出量は報告によって変動するが、Gerberら（2013）は3.5 G（10^9）t CO_2eq（二酸化炭素換算）/年と試算し、これは人為的に排出される温室効果ガスの7.1%に相当する[2)]。その内訳は消化管発酵由来メタンが最も高く（2.7 Gt CO_2eq/年）、次いで家畜排せつ物由来メタン・一酸化二窒素（0.7 Gt CO_2eq/年）、およびエネルギー消費由来二酸化炭素（0.1 Gt CO_2eq/年）となっている[2)]。このため、家畜生産からの温室効果ガス排出を抑制するためには消化管発酵由来メタンの抑制が不可欠である。

消化管発酵由来メタンの国別排出量は家畜飼養頭数の多いインドやブラジルで最も高く、ニュージーランドやアイルランドのような他産業に対して畜産業の規模が大きい国では、自国内の温室効果ガス排出量に占める消化管発酵由来メタン排出の割合が高い。特にニュージーランドでは総排出量の35%が消化管発酵由来メタンとなっており[3)]、約束草案作成に当たって、消化管発酵由来メタンに対して意欲的な削減目標を設定せざるを得ない。また、同国のような畜産物が国の重要輸出産品のひとつである国では、畜産物生産が温室効果ガス排出源として批判を受けることは回避したいものと考えられる。

開発途上国では2015年から2030年の間の牛肉および生乳生産量の増加率はそれぞれ、34%および40％と予測されている一方で、先進国ではそれぞれ0.4%および7%と予測されており、開発途上国の予測増加率は先進国よりも極めて高い[4)]。このため、開発途上国では少なくとも現在より温室効果ガス排出量を増やさない努力が必要である。

日本の2015年における温室効果ガス排出量は1.3 Gt CO_2eqであり、その内訳はエネルギー89％、工業プロセス及び製品の使用7％、農業2.5％、廃棄物1.6％、間接二酸化炭素0.2％となっている（土地利用、土地利用変化及び林業部門を除く）[5)]。消化管発酵由来メタン排出量は7,300 k（10^3）t CO_2eqであり、日本の温室効果ガス総排出量の0.55%を占めるに過ぎない[5)]。しかし、2016年に閣議決定された地球温暖化対策計画では長期的目標として2050年ま

Proceedings of Japanese Society for Animal Nutrition and Metabolism 61 (2): 21-31, 2017.
Potential for mitigating enteric methane emissions from cattle in Japan and Southeast Asia
Tomoyuki Suzuki, Japan International Research Center for Agricultural Sciences
Fuminori Terada, Graduate School of Agricultural Science, Tohoku University
Tomoyuki Kawashima, Faculty of Agriculture, University of Miyazaki

でに80%の温室効果ガス削減を目指すとしており、農業分野で水田に次いで2番目に大きな排出源である、消化管発酵についても抑制の取り組みは必要である。また、気候変動への対応だけでなく、畜産業の発展のためにも抑制の取り組みは必要と考えられる。日本では工業製品や食品へのカーボンフットプリント（CFP）表示の取り組みが緒についたばかりであるが、地球温暖化対策計画でもCFPの普及・促進を目的とした施策を実施するとされており、今後消費者の関心も高まっていくことが期待される。仮に諸外国の畜産由来温室効果ガス抑制への取り組みが大きく進む一方で、日本の対処が遅れた場合、輸入畜産物の温室効果ガス排出量が国産畜産物よりも低くなり、消費者が温暖化緩和の観点から、輸入畜産物を選択する時代が到来するかもしれない。日本に関しては、温室効果ガス抑制への取り組みは気候変動緩和への寄与に加え、自国の畜産業を守り、発展に寄与するために不可欠である。

国際農林水産業研究センターでは国際共同研究プロジェクト「開発途上地域農業の温室効果ガス排出抑制とリスク回避技術の開発」において、東南アジアにおける消化管発酵由来メタン、および糞尿処理過程におけるメタン・一酸化二窒素排出抑制を目的とした共同研究をタイおよびベトナムで行っている。気候変動問題は地球規模の課題であり、日本国内における消化管発酵由来メタン排出抑制のための研究は国内の排出抑制に資するだけでなく、抑制技術開発に十分な資源を投入できない開発途上国における排出抑制に貢献できる。そこで、本報告では日本および東南アジアの開発途上国におけるウシからの消化管発酵由来メタン排出抑制の可能性について検討する。

2. 日本における乳用牛からの消化管発酵由来メタン排出抑制の可能性

地球温暖化対策計画（2016年閣議決定）では約束草案の目標達成のための具体的な取り組みが明記された。日本の温室効果ガス排出量の約９割を占めるエネルギー起源二酸化炭素については25%減、メタン排出量については2013年度実績の36,000 kt CO_2eqから2030年度には12.3%減の31,600 kt CO_2eqにすることを目標としている。農業分野では水田からのメタン排出を640~2,430 ktCO2eq削減することを目指しており、消化管発酵由来メタンについては削減目標が設定されていない。また、農林水産省地球温暖化対策計画（2017年3月決定）では、畜産分野での排出削減対策について、温室効果ガス排出の少ない家畜排せつ物処理方法の普及、およびアミノ酸バランス改善飼料の給餌の普及が目標として掲げられているが（目標値はなし）、消化管発酵由来メタンに関しての目標は定められていない。ここでは、約束草案目標年の2030年における消化管発酵由来メタン排出量を試算し、排出抑制対策の導入によってどの程度抑制が可能かを乳用牛について検討する。

2013年の乳牛飼養頭数、乳量およびメタン排出量は日本国温室効果ガスインベントリ報告書[5)]を用いた。泌乳牛の排出係数および飼養頭数は他の区分のウシよりも高いため、泌乳牛のメタン排出量は最も高く、全体の7割を占めていた（表1）。

家畜改良増殖目標[6)]では、2025年における目標頭数を1,330千頭としており、これと現在の頭数から変化率を求め、2030年の飼養頭数を1,300千頭（うち2歳以上の雌牛920千頭）とした。また、同目標では2025年における目標個体乳量の上限を9,000 kg/年としており、これを2030年の目標個体乳量とした。この目標頭数と個体乳量の積から、2030年に生産される生乳は6,536 ktと算出でき、以下のどのケースでもこの総乳生産量は変わらないことを前提とした。家畜改良増殖目標[6)]では牛乳・乳製品の需要動向に即した生産を行うことを目指して個体乳量、飼養頭数の数値目標を設定している。その結果、近年の日本の生乳生産量は減少傾向にあるが、目標乳量と頭数から求めた2030年予測生乳生産量は2013年比8.4%増となった。

生産総乳量6,536 kt/年を、個体乳量が2013年レベルの泌乳牛で賄うと仮定するケースを成り行きケースとした（表中の「成り行き」）。成り行きケースでは、生産総乳量を達成するために泌乳牛が増えることとなり、これにともなって他の区分のウシを2013年と同じ構成比で増加するように調整した。その結果、ウシの総頭数が増えるため、排出量は2013年比8.0%増の151 kt/年となった。

表1. 日本の乳用牛における2013年、および2030年ケース別[1]飼養頭数、乳生産量および推定消化管発酵由来メタン排出量

		頭数	排出係数	排出量		乳生産量	
		千頭	kg/頭/年	kt/年	g/kg 乳量	kg/頭/305日	kt/年
2013年	泌乳牛	773	127.6	98.6	16.4	7,800	6,029
	乾乳牛	185	88.7	16.4			
	育成牛（<2歳,≥7ヵ月）	328	68.2	22.4			
	育成牛（3-6ヵ月）	73	34.6	2.5			
	育成牛（<3ヵ月）	36					
	合計	1,395		139.9			
2030年	泌乳牛	838	127.6	106.9	16.4	7,800	6,536
成り行き	乾乳牛	206	88.7	18.3			
	育成牛（<2歳,≥7ヵ月）	341	68.2	23.2			
	育成牛（3-6ヵ月）	76	34.6	2.6			
	育成牛（<3ヵ月）	38					
	合計	1,499		151.0			
	変化率（2013年比,%）	+7.4		+8.0			
2030年	泌乳牛	838	121.2	101.5	15.5	7,800	6,536
添加物使用	乾乳牛	206	81.6	16.8			
	育成牛（<2歳,≥7ヵ月）	341	62.7	21.4			
	育成牛（3-6ヵ月）	76	31.8	2.4			
	育成牛（<3ヵ月）	38					
	合計	1,499		142.2			
	変化率（2013年比,%）	+7.4		+1.6			
	変化率（成り行き比,%）	0		-5.9			
2030年	泌乳牛	838	127.6	106.9	16.4	7,800	6,536
平均産次増	乾乳牛	206	88.7	18.3			
	育成牛（<2歳,≥7ヵ月）	297	68.2	20.2			
	育成牛（3-6ヵ月）	66	34.6	2.3			
	育成牛（<3ヵ月）	33					
	合計	1,440		147.7			
	変化率（2013年比,%）	+3.2		+5.6			
	変化率（成り行き比,%）	-3.9		-2.2			
2030年	泌乳牛	726	131.3	95.4	14.6	9,000	6,536
個体乳量向上	乾乳牛	174	88.7	15.4			
	育成牛（<2歳,≥7ヵ月）	296	68.2	20.2			
	育成牛（3-6ヵ月）	66	34.6	2.3			
	育成牛（<3ヵ月）	33					
	合計	1,295		133.3			
	変化率（2013年比,%）	-7.2		-4.7			
	変化率（成り行き比,%）	-13.6		-11.7			
2030年	泌乳牛	726	124.8	90.6	13.9	9,000	6,536
複合	乾乳牛	174	81.6	14.2			
	育成牛（<2歳,≥7ヵ月）	258	62.7	16.2			
	育成牛（3-6ヵ月）	57	31.8	1.8			
	育成牛（<3ヵ月）	28					
	合計	1,244		122.8			
	変化率（2013年比,%）	-10.8		-12.2			
	変化率（成り行き比,%）	-17.0		-18.7			

[1] 各ケースの条件については本文参照のこと。

この成り行きケースで、飼料あるいは飼料添加物を用いたメタン排出係数低減による影響を試算した（表中の「添加物使用」）。飼料あるいは飼料添加物によるメタン排出抑制効果の文献値を表2 に示した。飼料添加物による排出抑制に関する報告は数多くみられるが、現時点、あるいは近い将来、賦存量

表2. 消化管発酵由来メタン抑制効果が認められる飼料および添加物の例[1]

	飼料および添加物	添加率[2]	抑制率[3]	文献番号
泌乳牛				
ホルスタイン種	硝酸塩	6.6%（NO_3として）	15.4%	7
ホルスタイン種	ホミニーフィード	27%	11.6%	8
ホルスタイン種	DDGS	30%	8.3%	9
ホルスタイン種	カシューナッツ殻液（CNSL）	4g CNSL/100kg体重	6.0%	10
ホルスタイン種	カノーラ油粕＆ホミニーフィード	それぞれ12%および14%	4.8%	8
非泌乳牛				
ホルスタイン種雌牛	カシューナッツ殻液	4g CNSL/100kg体重	19.3%	11
黒毛和種雌牛	生米ヌカ	12%	15.3%	12
ホルスタイン種去勢牛	ユッカ抽出液	1%（sarsaponinとして）	12.8%	13
タイ在来種雄牛	カポック種子	1g/kg体重	12.5%	14
ライシン種雄牛	ココナッツ油	0.63%	12.0%	15
黒毛和種雌牛	ビール粕	12%	7.7%	12

[1]賦存量や価格面から生産現場での使用に実現性がある添加物について、ウシを用いた試験で生産性(摂取量、消化率、乳生産成績等)に負の影響が見られなかった試験結果を抽出。
[2]特記がなければ給与飼料中の対象物の添加率（乾物ベース）。
[3]乾物当たりメタン排出量の対照区に対する低下率。

や価格の観点から、生産現場での使用に実現性がある飼料あるいは添加物を扱った報告のうち、ウシを用いた試験で、生産性あるいは生産性に影響する指標（乾物摂取量、消化率、代謝率等）に負の影響が見られなかった試験結果を抽出した。表中の泌乳牛および非泌乳牛における最も低い抑制率は、様々な条件下にある生産現場において実現が可能な抑制率と考え、泌乳牛および非泌乳牛のメタン排出抑制率をそれぞれ、5%および8%とした。その結果、排出係数の低下により、排出量は成り行きケース比5.9%減となったが、これは添加物使用による抑制効果が5.9%であることを示す。一方で、添加物使用によっても2013年排出量よりは1.6％高い結果となった。

家畜改良増殖目標[6)]では生涯生産性の向上にも言及しているが、現在の平均産次数2.7産（2014年）が統計[16)]のある1985年のレベル（3.1産）まで延長したと仮定したときのメタン排出に及ぼす影響を検討してみる（表中の「平均産次増」）。佐々木ら（2005）[17)]のシミュレーションから、群平均産次が2.7産から3.1産に延長することにより、育成牛飼養頭数は13%削減できることが試算できる。この育成牛削減による2030年成り行き比排出抑制率は2.2%であり、これは平均産次が0.4産伸びることによるメタン排出抑制効果が2.2%であることを示す。

個体乳量が向上し、2030年に家畜改良増殖目標[6)]にある個体乳量9,000 kg/年を達成した場合（表中の「個体乳量向上」）、泌乳牛の乾物摂取量は、成り行きケースに比べ増加するため排出係数も増加する。一方で成り行きケースに比べ泌乳牛の飼養頭数は減少し、これにともない他の区分のウシも減少するため、総排出量は2013年比4.7%の減少となり、個体乳量向上による排出抑制効果は11.7%と試算された。

最後に、ここまで検討してきた飼料あるいは飼料添加物使用、平均産次数向上、および個体乳量増加による抑制の取り組みすべてを実施した場合の排出量は2013年の12.2%減となり、複合抑制の効果は18.7%と試算された（表中の「複合」）。本検討での条件下でメタン排出抑制策を比較すると個体乳量向上が最も高い抑制率を示した。これは個体乳量向上による乳生産当たりメタン排出の低下に加え（2030年成り行きと個体乳量向上ケースでそれぞれ、16.4および14.6 g/kg乳量）、全体の飼養頭数が抑制されることによるものである。日本の個体乳量は1975年から2015年の間に67%増加してきたところであり、この期間も個体乳量の向上はメタン排出抑制に貢献してきたものと考えられるが、今後も個体乳量の向上は抑制策の一つとして重要である。

3. 日本における肉用牛からの消化管発酵由来メタン排出抑制の可能性

2013年の排出量および関連する統計量は日本国インベントリー[5]を用いた。2030年の頭数予測は家畜改良増殖目標[6]の2025年目標総頭数2,520千頭（うち肉専用種1,860千頭）より、2013年飼養頭数からの変化率を求め、2030年の総頭数を2,499千頭（うち肉専用種1,918千頭）とした。これに2013年の肉専用種および乳用種それぞれの区分別頭数比を乗ずることにより、各区分の飼養頭数を求めた（表3; 表中の「2013年」）。なお、家畜改良増殖目標[6]では、乳用後継牛の不足を生じさせない範囲で和牛子牛生産を拡大するとしており、このことから2030年は2013年に比べ肉専用種頭数は多く、乳用種は少なくなっている。この条件でメタン排出量を推定すると、総頭数は2030年には減少すること、加えて排出係数の大きい乳用種の頭数が減ることから2030年の排出量は2013年の5.0%減となった（表中の「2030年改良増殖目標」）。これに、メタン排出抑制効果を持つ飼料添加物を3ヵ月齢以上のウシ全頭に使用した場合、表2の非泌乳牛における最も低い抑制率より、飼料添加物による排出係数低下を8%とすると、総メタン排出量は2030年増殖目標の8%減となり、2013年の12.6%減となった（表中の「2030年目標+添加物」）。本報告ではすべての区分のウシで一律排出係数8%減としたが、表2の非泌乳牛における試験には肥育後期のような粗飼料割合が低い条件下での検討は含まれていない。これを考慮すると総排出量は本試算よりも高くなると考えられる。一方で、良質赤身肉生産を意図した早期出荷や肥育期間短縮といった、生産期間短縮に寄与する技術導入による、メタン排出抑制効果は評価していないため、これを考慮すればさらに排出量を抑制できる可能性がある。

4. 東南アジアの消化管発酵由来メタン排出の概要

東南アジアでの排出抑制の検討にあたっては、これまで当センターが共同研究を行ってきたタイの乳用牛および肉用牛に着目した。東南アジアにおけるタイの酪農および牛肉生産の位置づけについて表4に示した。タイの年間生乳生産量はミャンマーに次いで東南アジア2位であるが、生産量はミャンマーの半分程度と推定されている。一方で、ミャンマーの個体乳量は746 kg/年と見積もられており[18]、ホ

表3. 日本の肉用牛における2013年、および2030年ケース別[1]飼養頭数および推定消化管発酵由来メタン排出量

	2013年			2030年改良増殖目標			2030年目標+添加物		
	頭数 千頭	排出係数 kg/頭/年	排出量 kt/年	頭数 千頭	排出係数 kg/頭/年	排出量 kt/年	頭数 千頭	排出係数 kg/頭/年	排出量 kt/年
繁殖雌牛 ≥1歳	568	57.0	32.4	635	57.0	36.2	635	52.4	33.3
繁殖雌牛 <1歳, ≥7ヵ月	14	53.8	0.8	16	53.8	0.8	16	49.5	0.8
繁殖雌牛 3-6ヵ月齢	9	30.5	0.3	10	30.5	0.3	10	28.1	0.3
繁殖雌牛 <3ヵ月	5			6			6		
肥育牛 和牛 雄 ≥1歳	381	68.5	26.1	426	68.5	29.2	426	63.0	26.8
肥育牛 和牛 雄 <1歳, ≥7ヵ月	115	64.5	7.4	129	64.5	8.3	129	59.3	7.6
肥育牛 和牛 雄3-6ヵ月齢	77	30.6	2.4	86	30.6	2.6	86	28.2	2.4
肥育牛 和牛 雄 <3ヵ月	38			42			42		
肥育牛 和牛 雌 ≥1歳	328	51.9	17.0	367	51.9	19.0	367	47.7	17.5
肥育牛 和牛 雌 <1歳, ≥7ヵ月	91	48.7	4.4	102	48.7	5.0	102	44.8	4.6
肥育牛 和牛 雌3-6ヵ月齢	60	27.3	1.6	67	27.3	1.8	67	25.1	1.7
肥育牛 和牛 雌 <3ヵ月	30			34			34		
肥育牛 乳用種 ≥7ヵ月	639	75.6	48.3	425	75.6	32.1	425	69.6	29.6
肥育牛 乳用種3-6ヵ月齢	142	41.5	5.9	94	41.5	3.9	94	38.2	3.6
肥育牛 乳用種 <3ヵ月	71			47			47		
合計	2,568		146.6	2,499		139.3	2,499		128.2
変化率（対2013年,%）				-2.7		-5.0	-2.7		-12.6
変化率（対2030年増殖目標,%）									-8.0

[1] 各ケースの条件については本文参照のこと。

表4. 東南アジア[1]における2014年の生乳生産量、牛枝肉生産量および一人当たり名目国内総生産（GDP）

	生乳生産量[2], t	牛枝肉生産量[2], t	一人当たり名目 GDP[3], USD
カンボジア	24,607	69,205	1,096
インドネシア	800,749	497,669	3,534
ラオス	7,639	30,131	1,718
マレーシア	75,270	27,462	11,009
ミャンマー	2,164,832	232,804	1,275
フィリピン	20,010	201,390	2,844
タイ	1,067,452	163,264	5,921
ベトナム	549,533	292,501	2,047

[1] シンガポール、ブルネイ、東ティモール除く。
[2] 文献4より。
[3] IMF データーベース (https://www.imf.org/en/Data) より。

ルスタインあるいはその交雑種を導入した近代的な酪農が主体の国の中ではタイの乳生産量が域内で最も高いものと考えられる。肉用牛生産について、タイの牛枝肉生産量は域内で5位となっている。一人当たり名目国内総生産はマレーシアに次いで2位であり、畜産業への投資が比較的行いやすい環境にある。

アジアにおける消化管発酵由来メタン排出量を国別に比較すると、インドをはじめとする南アジアおよび中国の排出量が突出して高く、インドおよび中国のみでアジアの消化管発酵由来メタン排出量の6割を占めている（図1）。東南アジアについてみてみると、ミャンマーおよびインドネシアの排出量が他国よりも大きいが、東南アジア諸国の消化管発酵由来メタン排出総量はアジア全体の1割程度を占めるにすぎない。農業分野における主要な排出源である水田由来メタンについては、やはり中国およびインドの排出量が大きいが、そのアジアにおける総排出量への寄与は、消化管発酵由来メタンよりも低く45%程度である。一方で、東南アジア諸国からの水田由来メタン排出はアジア全体の43%を占める。水田からのメタン排出量には主に土壌中の易分解性有機物量および酸化還元電位が影響するため、稲ワラ施用の中止、および一定期間水田を排水する間断灌漑（中干し）がメタン排出抑制に効果があるとされている[5)]。東南アジアでは稲ワラおよび米ヌカ等の水稲生産から排出される副産物は主要な飼料資源のひとつであるが、メコンデルタにおける調査例[19)]にもあるように、稲ワラの大部分はすき込みあるいは焼却されていると考えられている。このため、今後も増加すると考えられる畜産物需要に対して、未利用稲ワラの飼料利用は不可欠と考えられ、環境的側面からは水田および畜産トータルでの温室効果ガス排出抑制を検討する必要がある[20)]。

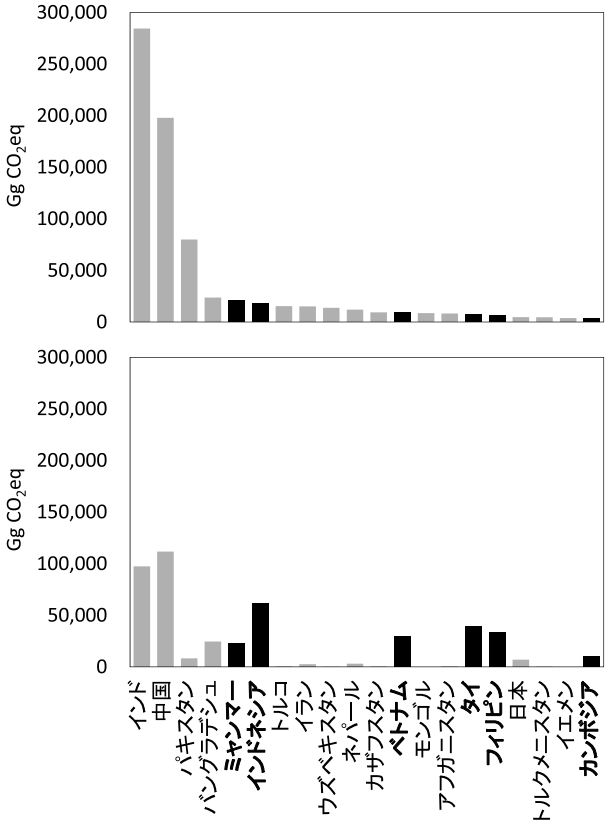

図1. 2013年のアジアにおける消化管発酵由来メタン排出上位20ヵ国の排出量（上段）および水田からのメタン排出量（下段）
太字は東南アジア諸国。文献18より作図

5. タイにおける乳用牛からの消化管発酵由来メタン排出量の推移

タイにおける泌乳牛からのメタン排出量の経年推移をタイで得られた予測式と統計データーから推定

表5. タイにおける泌乳牛飼養頭数、乳生産量および推定消化管発酵由来メタン排出量

	頭数[1]	生乳生産量[1] kt	個体乳量[1] kg/日	排出量[2] g/kg乳量	排出量[2] kt/年
1982年	13,770	27	5.4	27.5	1
1987年	36,200	79	6.7	25.4	2
1991年	59,850	147	6.8	25.3	4
1996年	117,130	328	7.8	23.7	8
2005年	296,472	781	11.6	17.7	14
2009年	293,287	883	12.3	16.5	15
2012年	295,634	1,064	12.5	16.2	17

[1] 文献24より。
[2] 文献22の予測式より推定。

した。Odai ら（2010）はタイにおいてマスク法[22]により実施された乳牛（ホルスタインとタイ在来種との交雑種）のエネルギー出納試験結果を用い（n = 20）、個体乳量1 kgあたりのメタン排出量（Y, L/kg）を個体乳量（X, kg/日）から予測する式を示した（$Y = -2.22X + 50.40$; $R^2 = 0.39$, $P = 0.003$）[21]。この予測式と個体乳量および総生乳生産量の統計データー[23]を用いてメタン排出量を推定した（表5）。タイにおける酪農の近代化はデンマークおよびドイツの支援により1960年代から始まったが、1991年以降に生乳生産量が急速に増加し、個体乳量については1996年以降大きく増加した。このため個体乳量1kgあたりのメタン排出量は現在まで低下し続けていると試算されたが、全体の飼養頭数と乳生産量の増加により2012年における泌乳牛全体からのメタン排出量は1982年の17倍と推定された。この30年の間、産乳能力が向上しなかった場合、すなわち個体乳量が1982年のままで各年の総乳量を生産した場合、産乳能力が向上した場合との差は年々大きくなる（図2）。このことは、総排出量は年々増えているが、家畜の改良および飼養管理技術向上により排出量の増加が抑制されてきたことを示している。2012年におけるメタン排出量と、個体乳量向上がない場合の2012年メタン排出量との差から、30年間の家畜改良・管理技術向上による排出抑制効果は41%であったと言える。現在（2012年）の平均個体乳量は12.5 kg/日であり、交雑種ではあるが乳生産能力にはまだ向上の余地があるものと考えられ、今後も家畜改良・管理技術向上によるメタン排出抑制は期待できるだろう。一方で、開発途上国では2015年から2030年の間の生乳生産量の増加率は34%と予測されており[4]、タイでも同程度の増加となる場合、総排出量を現状レベルよりも下げることは難しいだろう。

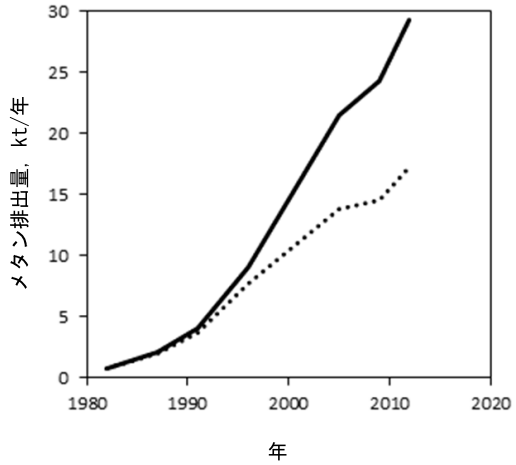

図2. タイにおける泌乳牛からの推定消化管発酵由来メタン排出量(点線)および泌乳牛の個体乳量が1982年レベルのまま各年の生産乳量を達成した場合の推定消化管発酵由来メタン排出量(実線)

6. タイにおける肉用牛からの消化管発酵由来メタン抑制の可能性

東南アジアでの抑制の可能性について検討するために、タイを例として抑制効果の検討を試みた。Boonyanuwat ら（2013）はタイにおける肉用牛の飼養体系を、放牧を主体とする粗放的飼養、舎飼でより栄養価の高い飼料を給与する集約的飼養、および放牧と舎飼を併用する中間的飼養の３つに区分し、約９割の肉用牛は粗放的に飼養されており、粗放的飼養下での日増体量は中間的飼養の半分、集約的飼養の28%程度と推定している（表6; 表中の「2014年」）[24]。現在のメタン排出量を推定するにあたり、各飼養区分の飼養頭数は、各飼養区分構成比[24]を

表6. タイの肉用牛における2014年、および2030年ケース別[1]飼養頭数、日増体量および推定消化管発酵由来メタン排出量

		頭数 千頭	排出係数 kg/頭/年	排出量 kt/年	日増体量 kg/頭/日	日増体量 t/日
2014年	粗放的飼養	4,153	37	155	0.27	1,109
	中間的飼養	99	47	5	0.59	58
	集約的飼養	60	57	3	0.95	57
	合計	4,312		163		1,224
2030年 成り行き	粗放的飼養	5,544	37	206	0.27	1,480
	中間的飼養	132	47	6	0.59	78
	集約的飼養	81	57	5	0.95	77
	合計	5,757		217		1,635
	変化率(2014年比,%)	+33.5		+33.5		
2030年 添加物使用	粗放的飼養	5,544	37	206	0.27	1,480
	中間的飼養	132	43	6	0.59	78
	集約的飼養	81	52	4	0.95	77
	合計	5,757		216		1,635
	変化率(2014年比,%)	+33.5		+33.0		
	変化率(成り行き比,%)	-		-0.4		
2030年 飼養管理変化	粗放的飼養	2,772	37	103	0.27	740
	中間的飼養	1,393	47	65	0.59	818
	集約的飼養	81	57	5	0.95	77
	合計	4,246		173		1,635
	変化率(2014年比,%)	-1.5		+6.5		
	変化率(成り行き比,%)	-26.2		-20.2		
2030年 復合	粗放的飼養	2,772	37	103	0.27	740
	中間的飼養	1,393	43	60	0.59	818
	集約的飼養	81	52	4	0.95	77
	合計	4,246				1,635
	変化率(2014年比,%)	-1.5		+3.1		
	変化率(成り行き比,%)	-26.2		-23.0		

[1]各ケースの条件については本文参照のこと。

現在（2014年）の総飼養頭数4,312千頭[25]に乗ずることにより求めた。総エネルギー摂取量および日増体量についてはBoonyanuwatら（2013）の値を用いた[24]。タイおよびベトナムで実施したヘッドボックス法[26, 27]による呼吸試験から得られたメタン転換係数（総エネルギー摂取量当たりの排出されたメタンのエネルギー）344例（個体ベース）について、給与飼料中の粗飼料割合（乾物ベース）によって低粗飼料区（≤ 33%; n = 83）、中粗飼料区（34 – 67%; n = 73）および高粗飼料区（≥ 68%; n = 188）の3つにグループ分けした。この3グループがBoonyanuwatら（2013）の集約的飼養、中間的飼養および粗放的飼養にそれぞれ対応するものと仮定した[24]。各区分の総エネルギー摂取量とメタン転換係数から2014年のメタン排出係数（1年1頭当たりメタン排出量）を求めたところ、集約的飼養、中間的飼養および粗放的飼養でそれぞれ、37, 47および57 kg/頭/年となった（表中の「2014年」）。その結果、全肉用牛からのメタン排出量は163 kt/年となり、そのうち95%が粗放飼養下の肉用牛由来となった。

FAO（2002）は、開発途上国における2015年および2030年の牛肉生産量は346,000および484,000 ktとなり、この期間で生産量は33.5%増加することを予測している[4]。この予測をタイの肉牛生産にあてはめ、飼養形態の構成比、ウシ品種、日増体量に変化がなく、2030年の牛肉生産量が2014年比33.5%増加した場合、タイにおける肉牛飼養頭数は5,757千頭となり、メタン排出量は現在の34%増の217 kt/年と試算された（表中の「成り行き」）。これを2030年排出量の成り行きケースとし、飼料あるいは飼料添加物の使用、あるいは飼養管理方法の変更による排出量抑制効果を検討する。

飼料あるいは添加物給与による抑制効果検討にあたり、表2の非泌乳牛で最も低い抑制率より、2030年の成り行きケースにおける排出係数を8%減として総排出量を試算した(表中の「添加物使用」)。なお、粗放的飼養では粗飼料以外の飼料給与はほとんどないと考えられるため、中間的飼養および集約的飼養でのみ添加物を給与し、排出係数が8%低下するものとした。その結果、肉用牛の大部分は粗放的飼養に区分されているため、総排出量は2030年の成り行きケースに比べて0.4%の減少としかならなかった。

次に、2030年の成り行きケースの牛肉生産量は変わらず、飼養管理構造が変化することを検討してみる（表中の「飼養管理変化」)。すなわち、日増体量の合計1,635 tを維持したまま、粗放的飼養のウシ飼養頭数が半減し、中間的飼養が増加した場合を想定すると（集約的飼養は変化なしとする)、日増体量の高い、中間的飼養の飼養頭数が増えるため、全体の頭数は4,246千頭と、成り行きケースよりも飼養頭数が少なくなる。この時の総排出量は173 kt/年であり、2011年からの増加率は成り行きケースに比べ大きく抑制されて6.5%の増加、2030年の成り行きケースと比べて20.2%減となる。この飼養構成において、中間および集約的飼養牛に対して排出係数抑制効果8%の添加物を使用した場合（表中の「複合」)、2030年成り行きケースの23.0％減、2011年比3.1%増となった。すなわち､肉用牛飼養の集約化(生産性向上）と添加物の使用は23.0%のメタン排出抑制効果があることを示すが、それでも2011年の排出レベルよりは高くなってしまう。生産性向上のためには高栄養な飼料が必要となるが、高栄養価の牧草に加えて、精米、デンプン抽出、製糖、搾油といった農業・食品製造過程で産出される副産物の有効利用が不可欠である。副産物は一般的に栄養的な偏りがあることから栄養価の確認および適切な給与技術の提示が必要である。また、水分、糖質、あるいは脂質等を高含量に含むものについては貯蔵技術の開発が必要である。

7. まとめ

本報告で試みた消化管発酵由来メタン排出量の将来予測と抑制の可能性から、日本では頭数の減少と抑制技術適用、加えて乳牛の産乳能力向上により現在よりも排出量を減らすことができると考えられた。畜産関係者の相当な努力が必要であるが、日本における消化管発酵由来メタン排出抑制は、現在利用可能な技術で、必要とされる生産量を維持しながら、乳用牛および肉用牛でそれぞれ12％および13％抑制を達成することは可能であると考えられる。日本の温室効果ガスインベントリは1990年から始まったが、乳用牛、肉用牛でそれぞれ上記の削減が達成できれば1990年以降、最も低い排出量となる。それでも消化管発酵由来メタンで日本の総温室効果ガス削減目標と同じ26%減を達成することは困難である。タイでは乳用牛、肉用牛ともに、生産性向上による抑制効果は大きいが、今後も頭数の増加が予想され、2030年の排出量を現在よりも減らすことは難しいものと考えられた。

日本におけるさらなる抑制や、タイ等の東南アジアにおける現状排出量以下への抑制のためには、革新的な技術開発、消化管発酵由来メタンに限らない畜産業全体からの抑制の視点、生産性向上の追求、そして開発された技術の国外展開が不可欠である。例えば、新たな抑制材として3-nitrooxypropanol（3NOP）製剤の研究開発が進んでいる。3NOPは反すう胃内におけるメタン生成の最終段階であるメチル基転移反応を阻害するとされており、30%程度の排出抑制効果があるとされている[28)]。また、低メタン排出家畜育種[29)]や抗メタン細菌ワクチン[30)]といった新たなアプローチによるメタン排出抑制技術開発が進められている。

ウシ生産からの温室効果ガス排出源としては他に、糞尿由来メタンおよび一酸化二窒素、飼料畑からの一酸化二窒素放出、家畜・飼料生産に伴う化石燃料消費時の二酸化炭素排出等があげられる。消化管発酵由来メタン排出抑制効果の検討においては、糞尿由来温室効果ガス産生に影響を及ぼす有機物および尿素排出量も合わせて考慮すべきである。また、農業および食品副産物、および国内で生産された飼料の利用は、飼料生産に伴う温室効果ガス排出抑制に効果的であり、ライフサイクルアセスメントによる総合的な評価がなされている[31)]。

生産性向上による消化管発酵由来メタン排出抑制の観点から、研究においては従来の栄養分野のみでなく、管理、育種、環境、衛生等、分野を横断した取り組みが必要である。また、我が国では温暖化による家畜生産への負の影響が顕在化してきているが、家畜生産性低下はメタン排出量増加の要因となるため、温暖化適応策の技術開発においても開発された技術のメタン排出抑制効果を評価する必要がある。

気候変動問題は一国で完結する問題ではないため、得られた技術については、特に、今後の急速な排出量増加が予想される開発途上国での応用や展開が必要である。興味深い事例として、日本で開発されたカシューナッツ殻液によるメタン排出抑制技術のタイへの展開が試みられているところであるが[32]、開発途上国においては現地で安価に入手可能な資源を用いることが抑制技術普及の一つの条件と考えられる。

謝　辞

本論文執筆にあたり、国際農林水産業研究センターの宝川靖和サブプロジェクトリーダーからは有益なご助言を頂いたことをここに深謝いたします。

【引用文献】

1）IPCC. 2007. Climate Change 2007. The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (Eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

2）Gerber PJ, Steinfeld H, Henderson B, Mottet A, Opio C, Dijkman J, Falcucci A, Tempio G. 2013. Tackling climate change through livestock – A global assessment of emissions and mitigation opportunities. FAO, Rome.

3）Ministry for the Environment. 2017. New Zealand's Greenhouse Gas Inventory 1990–2014. New Zealand.

4）FAO 2002. World Agriculture: Towards 2030/2050-Interim Report. FAO, Rome.

5）国立環境研究所. 2017. 日本国温室効果ガスインベントリ報告書. 国立環境研究所.

6）農林水産省. 2015. 家畜改良増殖目標. 農林水産省. 東京.

7）van Zijderveld SM, Gerrits WJJ, Dijkstra J, Newbold JR, Hulshof RBA, Perdok HB. 2011. Persistency of methane mitigation by dietary nitrate supplementation in dairy cows. J. Dairy Sci., 94: 4028–4038.

8）Moate PJ, Williams SRO, Grainger C, Hannah MC, Ponnampalam EN, Eckard RJ. 2011. Influence of cold-pressed canola, brewers grains and hominy meal as dietary supplements suitable for reducing enteric methane emissions from lactating dairy cows. Anim. Feed Sci. Technol., 166–167: 254–264.

9）Benchaar C, Hassanat F, Gervais R, Chouinard PY, Julien C, Petit HV, Massé DI. Effects of increasing amounts of corn dried distillers grains with solubles in dairy cow diets on methane production, ruminal fermentation, digestion, N balance, and milk production. J. Dairy Sci., 96: 2413–2427.

10）真貝拓三・永西修・三森眞琴・長嶋協・小林泰男.2014. カシューナッツ殻液を利用した乳牛からのメタン低減技術. 栄養生理研究会報, 58: 45–52.

11）Shinkai T. Enishi O, Mitsumori M, Higuchi K, Kobayashi Y, Takenaka A, Nagashima K, Mochizuki M, Kobayashi Y. 2012. Mitigation of methane production from cattle by feeding cashew nut shell liquid. J. Dairy Sci., 95: 5308–5316.

12）永西修. 2002. 製造副産物の給与による家畜からのメタン産生抑制. 肉用牛からのメタン産生抑制技術の開発（プロジェクト研究成果シリーズ404）, 15–18. 農林水産技術会議. 東京.

13）Lila ZA, Mohammed N, Kanda S, Kurihara M, Itabashi H. 2003. Sarsaponin effects on ruminal fermentation and microbes, methane production, digestibility and blood metabolites in steers. Asian-Aust. J. Anim. Sci., 18: 1746–1751.

14）Kaewpila C, Suzuki T, Sommart K. 2015. Effect of whole kapok seed or chitosan supplementation on feed intake and methane emission of Thai native beef cattle fed rice straw. In Lu Y, Takehara K, Beynen AC, Yasuhiro K, Haron W (Eds.), Proceeding of the 5th International

Conference on Sustainable Animal Agriculture for Developing Countries (SAADC2015), 216. Rajamangala University of Technology Isan. Nakorn Ratchasima, Thailand.

15）Nguyen TKD, Nguyen VT, Cai Y, 2016. Effect of different levels of coconut oil supplementation to Lai Sind cattle diets. JIRCAS Working Report, 84: 26–30.

16）家畜改良事業団 2016. 平成２７年度乳用牛群能力検定成績のまとめ. 家畜改良事業団. 東京.

17）佐々木修・長嶺慶隆・石井和雄・武田尚人・古川力. 2005. 平均産次を上げるための牛群産次構成. 2004年研究成果情報. 畜産草地研究所. http://www.naro.affrc.go.jp/project/results/laboratory/nilgs/2004/nilgs04-08.html

18）FAO. (2017) FAO statistical databases. http://www.fao.org/faostat/en/#home

19）Hong Van N, Nga T, Arai H, Hosen Y, Chiem N, Inubushi K. 2014. Rice straw management by farmers in a triple rice production system in the Mekong Delta, Viet Nam. Trop. Agri. Develop., 58: 155 – 162.

20）宝川靖和. 2017. ベトナムメコンデルタにおける温室効果ガス排出削減技術と今後の展開. JATAFFジャーナル, 5: 5-9.

21）Odai M, Nonaka I, Sumamal W, Narmsilee R, Pholsen P, Chuenprecha T, Phromloungsri A, Khotprom N, Phaowphaisal I, Indramanee S, Suzuki T. 2010. Estimation of Methane Production by Lactating and Dry Crossbred Holstein Cows in Thailand. JARQ, 44: 429 – 434.

22）Kawashima T, Sumamal W, Terada F, Shibata M. 2001. Respiration trial system using ventilated flow-through method with face mask. JIRCAS Journal for Scientific Paper, 9: 54–57.

23）Langholz J, Holm J, Nissen N. 2016. 50 Years Dariy development in Northern Thailand. Trio Advertising & Media, Chiangmai, Thailand.

24）Boonyanuwat K, La VK, Sithambaram S, Widyawati Y. 2013. Improved inventory and mitigation of greenhouse gasses in livestock production in South East Asia. Livestock Emissions & Abatement Research Network (LEARN). New Zealand.

25）Osothongs M, Khemsawat J, Sarakul M, Jattawa D, Suwanasopee, Koonawawootrittriron. 2016. Current situation of beef industry in Thailand. In: Proceedings of International Symposium “Dairy cattle beef up beef industry in Asia: Improving productivity and environmental sustainability”. 5–8. Bangkok. Thailand.

26）Suzuki T, Phaowphaisal I, Pholsen P, Narmsilee R, Indramanee S, Nitipot P, Chaokaur A, Sommart K, Khotprom N, Panichpol V, Nishida T. 2008. In vivo nutritive value of Pangola grass （Digitaria eriantha） hay by a novel indirect calorimeter with a ventilated hood in Thailand. JARQ, 42: 123–129.

27）Sakai T, Kim Chi T, Nguyen Van T, Suzuki T, Hayashi K, Higuchi K. 2016. Ventilated hood system for measurement of enteric methane emissions in Vietnam. JIRCAS Working Report, 84: 10–14.

28）Hristov AN, Oh J, Giallongo F, Frederick TW, Harper MT, Weeks HL, Branco AF, Moate PJ, Deighton MH, Williams SRO, Kindermann M, Duval S. 2015. An inhibitor persistently decreased enteric methane emission from dairy cows with no negative effect on milk production. PNAS, 112: 10663–10668.

29）de Haas Y, Windig JJ, Calus MPL, Dijkstra J, de Haan M, Bannink A, Veerkamp RF. Genetic parameters for predicted methane production and potential for reducing enteric emissions through genomic selection. J. Dairy Sci., 94: 6122–6134.

30）Wedlock DN, Janssen PH, Leahy SC, Shu D, Buddle BM. 2013. Progress in the development of vaccines against rumen methanogens. Animal, 7: 244–252.

31）Ogino A, Sommart A, Subepang S, Mitsumori M, Hayashi K, Yamashita T, Tanaka Y. 2016. Environmental impacts of extensive and intensive beef production systems in Thailand evaluated by life cycle assessment. J. Cleaner Prod., 112: 22–31.

32）Konda S, Onodera R, Kanchanasatit E, Boonsaen P, Sawanon S, Nagashima K, Koike S, Kobayashi Y. 2016. Cashew nut shell liguid feeding for mitigating methane production in Thai local ruminants. In: Program book 6th Greenhouse Gas and Animal Agriculture Conference, 90. Melbourne, Australia.

Potency of ginkgo fruit for modulation of rumen microbiota and fermentation

Seongjin Oh, Yutaka Suzuki, Satoshi Koike and Yasuo Kobayashi*

(*Graduate School of Agriculture, Hokkaido University, Sapporo, 060-8589, Japan*)

Introduction

Enteric methane is produced by methanogenic archaea in gastrointestinal tract of ruminant animals during their feed utilization, which closely related to greenhouse gas emission and loss of energy in feed stuff[1]. Therefore, methane reduction has been attempted by the use of rumen modulating agents including antibiotics[2]. Although ionophore antibiotics have exhibited beneficial effect on rumen fermentation and animal performance, use of these additives have been gradually prohibited due to concerns on public health[3, 4]. The use of "natural" sources may not be subject to concerns raised regarding the overuse of chemically- and biologically- synthesized antibiotics including ionophores[5]. Many plant-derived compounds have been paid attention as a safer additive candidate for modulating rumen fermentation. Salicylate, its derivatives[6], flavonoids and terpenoids[7] have been reported to possess different extents of antimicrobial function.

Ginkgo is grown widely among Far East Asian countries such as China, Korea and Japan. Industrial uses of ginkgo are leaves for medicine (China) and nuts for food (Korea and Japan). Leaf extracts for medicinal use are even exported to European countries. Ginkgo fruit is a byproduct in the process of ginkgo nut separation (unsuitable for any uses due to its peculiar smell), yielding ca. 2,600 metric t/yr in Japan, accounted for 230% of nut production[8]. Therefore, biomass of ginkgo fruit is much smaller in comparison with the previously reported potent additive candidate cashew nut shell liquid (CNSL). In this regard, use of ginkgo fruit for feed additive might be limited locally. However, no evaluation has been made so far as to the functionality of ginkgo fruit as a feed additive candidate for ruminants. Although CNSL containing anacardic acid has been evaluated and found to be an effective rumen modifier in the previous studies[9, 10], its additive formula has not yet been widely available in the world and has not been fully appreciated on animal feed market. It is better to have alternatives to CNSL that should be a similarly effective plant source of anacardic acid. In this regard, ginkgo fruit is a suitable alternative candidate to be evaluated in the same manner as CNSL was done.

We describe here about the initial screening of potent cultivar of ginkgo for the use to modulate rumen fermentation, dose-response of rumen fermentation to ginkgo extract, microbial and fermentation changes in artificial rumen, and also the potency of ginkgo extract under various feeding conditions.

Ginkgo fruit extract and alkylphenols

Of six different ginkgo cultivars distributed in Japan, we chose 2 major cultivars of ginkgo, Kyuju (K) and Tokuro (T), as candidate materials based on availability from annual production. Fresh fruit after separation of the nut was obtained at a ginkgo farm in Sobue town, Aichi Prefecture, Japan, a major ginkgo-nut-producing area. For extraction, ginkgo fruit was suspended in of 99.5% ethanol for 48 h, centrifuged to obtain the supernatant, and then concentrated by a centrifugal evaporator. This stock extract was diluted with 99.5% ethanol to set each experimental dosage of ginkgo extract, expressed as percent (wt/vol) of

Proceedings of Japanese Society for Animal Nutrition and Metabolism 61 (2): 33-41, 2017.
*Correspondence to:
Yasuo Kobayashi, PhD
Email: kyas@anim.agr.hokudai.ac.jp

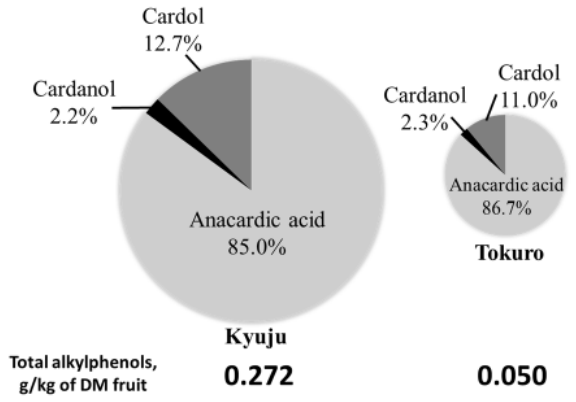

Fig. 1. Comparison between two different cultivars Kyuju and Tokuro in alkylphenolic compounds of ginkgo fruit. Alkylphenolics (anacardic acids, cardanol, and cardol) were quantified by HPLC [9].

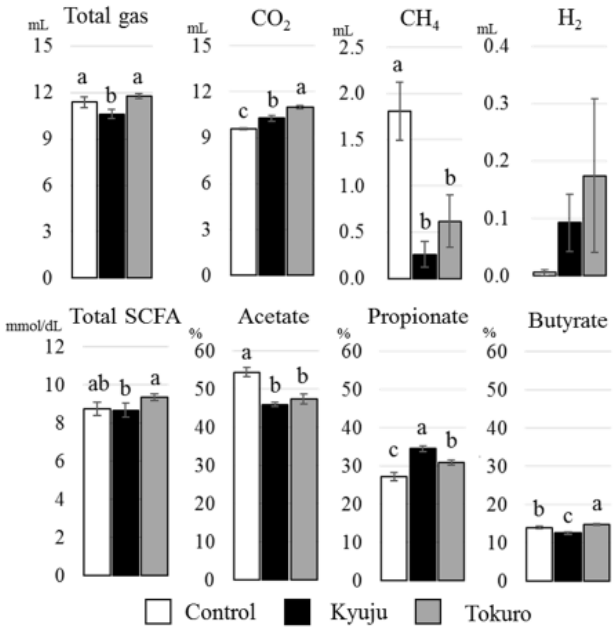

Fig. 2. Comparison between two different cultivars Kyuju and Tokuro in rumen gas and short chain fatty acid from cultures with ginkgo fruit extract. Ginkgo extract (1.6% fruit equivalent in final culture) was added to each treatment, while ethanol was to control. Bars with different superscripts differ (P<0.05).

ginkgo fruit (wet weight equivalent) in the final culture. The alkylphenols in 2 cultivars of ginkgo fruit are shown in Fig. 1. Both cultivars contained 3 types of anacardic acids, which were classified by length of the alkyl side chain and the number of double bonds (C13:0, C15:1, and C17:1). Ginkgo fruit also had cardanol (C15:1) and cardol (C15:1). The total content of these alkylphenols was more than 5 times higher in K than in T, although proportion of alkylphenols in each cultivar was similar. Thus, ginkgo fruit was confirmed to contain functional alkylphenolics as seen in CNSL.

Screening of more potent ginkgo cultivar

Batch culture studies were carried out to select more potent ginkgo cultivar as follows. Rumen fluid was diluted by a buffer in test tubes with a substrate that was a mixture of concentrate and hay (2.3:1). Ginkgo extract or an equal volume of ethanol was added to tubes and anaerobically incubated at 39°C for 24 h. For cultivar comparison, the dose of ginkgo was set at 1.6% in fruit equivalent. A comparison of the potency of the 2 ginkgo cultivars is shown in Fig. 2 . Although extracts from both cultivars greatly decreased methane production, K showed greater potential than T (86 vs. 66%). Total SCFA concentration was not changed by either ginkgo extract compared with the control. The concentration and molar proportion of acetate was decreased by K but not by T. Both ginkgo extracts enhanced propionate production. We observed lower butyrate production for K. Based on these observations, we selected K as the more potent additive for modulating rumen fermentation and used it for further study.

Dose response of rumen fermentation to ginkgo extract

The selected cultivar ginkgo K was evaluated for the dose response of rumen fermentation parameters, using 5 different doses (0, 0.8, 1.6, 3.2, and 6.4%), and results obtained are shown in Fig. 3 . Total gas production decreased at ≥3.2% supplementation. Methane production was linearly inhibited in a dose-dependent manner. Supplementation at >3.2% was quite effective for decreasing methane, but hydrogen accumulation occurred at ≥3.2%. Total SCFA concentration was not affected by the ginkgo extract. Acetate production linearly decreased, but propionate production linearly increased, depending on dose. The concentration and molar proportion of butyrate decreased at higher levels (≥3.2%). All parameters except CO_2 production showed a linear increase or decrease in dose response. Based on the observation of a decrease in total gas production and accumulation of hydrogen at

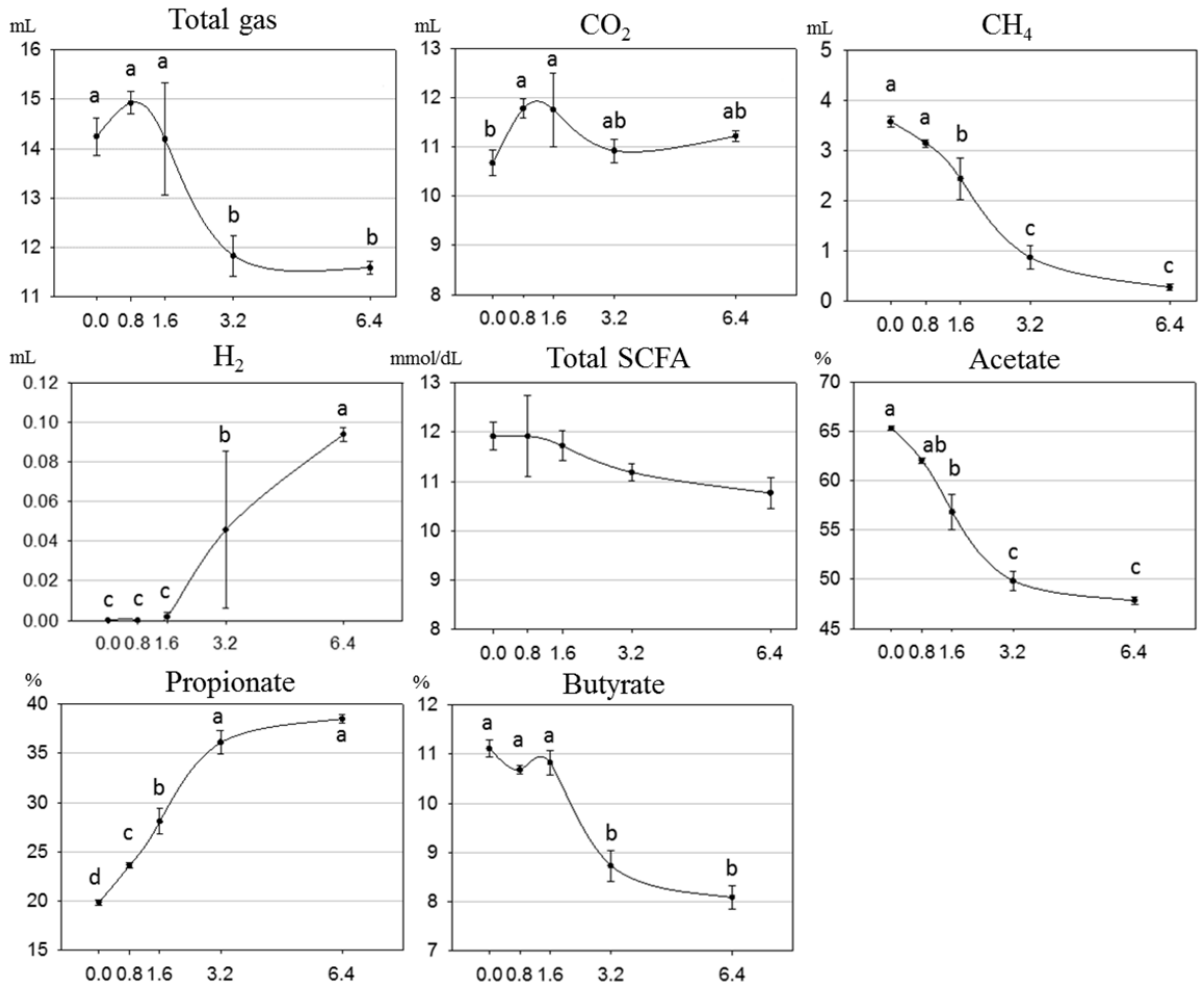

Fig. 3. Dose response of rumen fermentation parameters to selected ginkgo (Kyuju) extract supplementation (tested at 5 different levels that were 0, 0.8, 1.6, 3.2 and 6.4% of fruit equivalent). Dots with different superscripts differ (P<0.05).

≥3.2%, we selected the 1.6% dose of ginkgo fruit equivalent for further evaluation.

Evaluation of ginkgo extract by rumen simulation technique

Longer-term rumen responses to ginkgo extract were evaluated by the rumen simulation technique (RUSITEC) [11] that allows continuous fermentation under more practical condition. Rumen fermentation profiles and feed digestibility are shown in Fig. 4 . Total gas and CO_2 production was not affected, but methane production decreased by 47% when ginkgo extract was added. Ginkgo extract did not influence total SCFA concentration. The concentration and molar proportion of acetate were decreased by ginkgo extract, but the corresponding values of propionate were increased. Ruminal pH was significantly decreased. Ammonia concentration deceased, while disappearance of DM, NDF, and ADF were not affected by ginkgo extract.

Changes in bacterial and archaeal communities at different taxonomic levels with ginkgo extract supplementation are shown in Fig. 5 . For bacteria at the phylum level, the abundance of *Bacteroidetes* decreased with ginkgo extract, but the abundance of *Firmicutes* increased. The abundance of *Proteobacteria* was 4.6 times higher with ginkgo extract. More drastic change was seen for *Synergistes*, showing a 41-fold increase with ginkgo extract, of which *Pyramidobacter* was the main member. At the genus level, increased abundance with ginkgo extract was seen for specific groups related to succinate and propionate production, or both. These were *Succinivibrio*, *Ruminobacter*, *Selenomonas*,

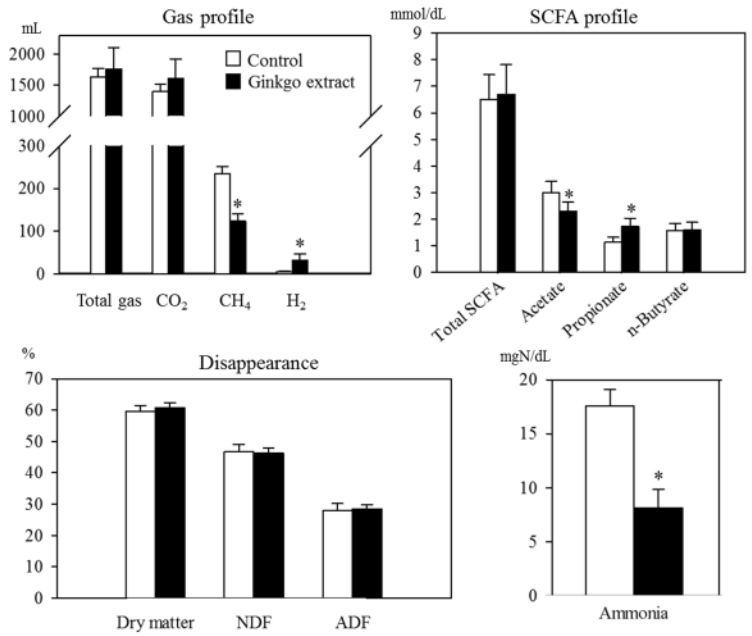

Fig. 4. Effect of ginkgo (Kyuju) extract supplementation on rumen fermentation profile in RUSITEC (rumen simulation technique). Ginkgo extract (1.6% fruit equivalent in final culture) was added to treatment, while ethanol was to control. Asterisk (*) indicates significant difference between control and treatment (P<0.05).

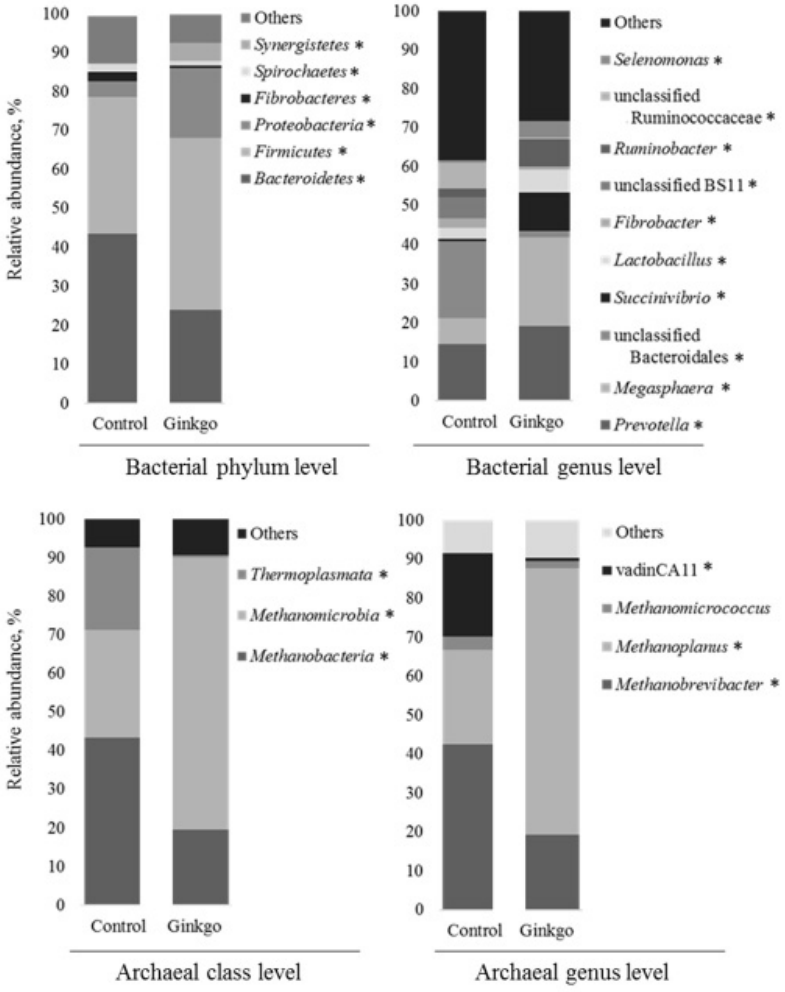

Fig 5. Effect of ginkgo extract supplementation on rumen microbiota in RUSITEC (rumen simulation technique) evaluated by MiSeq. Ginkgo extract (1.6% fruit equivalent in final culture) was added to treatment, while ethanol was to control. Asterisk (*) indicates significant difference between control and treatment (P<0.05).

Megasphaera, and *Prevotella*. At the same time, decreased abundance was seen for *Fibrobacter* and unclassified bacteria belonging to the *Bacteroidales*, *Clostridiales*, and *Ruminococcaceae*. The archaea community in the control comprised 3 classes: *Methanobacteria*, *Methanomicrobia*, and *Thermoplasmata*. By adding ginkgo extract, the community was changed toward more *Methanomicrobia* and less *Methanobacteria* and *Thermoplasmata*. The majority of each class were the genera *Methanobrevibacter*, *Methanoplanus*, and vadin CA11 (*Methanomassiliicoccaceae*). Thus, it is apparent that ginkgo fruit extract selectively inhibits rumen microbes, leading to fermentation changes.

Pure culture study: Minimum inhibitory concentration (MIC) test

We cultivated representative rumen bacteria to determine minimum inhibitory concentratin (MIC) of ginkgo K extract and of the purified phenolic compounds in ginkgo, and MIC values are shown in Fig. 6 . The bacterial response to ginkgo extract and anacardic acid was clearly divided into sensitive and insensitive groups. The sensitive group included *R. flavefaciens, R. albus, E. ruminantium, B. fibrisolvens, B. proteoclasticus, S. bovis, F. succinogenes, P. ruminicola, S. amyloytica, C. sticklandii, C. aminophilum,* and *P. anaerobius*. Insensitive species were *L. ruminis, S. dextrinosolvens, R. amylophilus, S. ruminantium,* and *M. elsdenii*. Hydrogen/formate producers and hyper ammonia-producing bacteria belonged to the sensitive group, but succinate- and propionate-producing (or both) bacteria were in the insensitive group. Purified cardanol and cardol, phenolic compounds present in ginkgo, did not show strong antibacterial activity to rumen bacteria except *C. sticklandii*. Thus, it is apparent that ginkgo extract and 3 anacardic acids show selective inhibition of bacterial growth at pure culture level, confirming that such selection occurs at mixed culture level in rumen simulation technique (see above).

Batch culture evaluation of ginkgo extract under different diet conditions

Effect of ginkgo extract supplementation on rumen fermentation was compared under five different forage to concentrate (F:C) ratios (Fig. 7) . Total gas production for each dietary substrate was not affected by ginkgo extract supplementation, except for the observed decrease with the 5:5 ratio diet. Methane decreased with ginkgo extract supplementation, with the greatest reduction found with the 5:5 ratio (41.9%); whereas the F:C ratio itself had no marked effect on methane production. Hydrogen was accumulated following ginkgo extract supplementation at 3:7, 5:5, 7:3 and 9:1 F:C ratios. Total SCFA concentration was not affected by ginkgo extract supplementation in each dietary substrate. However, total SCFA was clearly

Species		Gram-positive										Gram-negative						
		S. bovis	*L. ruminis*	*C. sticka ndii*	*C. amino philu m*	*P. anaer obius*	*E. rumin antiu m*	*B. proteo clasticu s*	*B. fibrisol vens*	*R. albus*	*R. flavefa ciens*	*F. succino genes*	*P. ruminic ola*	*S. amylol ytica*	*R. amylo philus*	*S. dextri nosol vens*	*S. rumin antiu m*	*M. elsde nii*
Gingko (%)		0.10	>1.61	<0.05	0.10	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	>1.61	>1.61	>1.61	>1.61
AA (μg/mL)	C13:0	12.5	>12.5	6.25	12.5	1.56	6.25	6.25	6.25	3.13	3.13	12.5	12.5	6.25	>12.5	>12.5	>12.5	>12.5
	C15:1	>12.5	>12.5	6.25	>12.5	6.25	3.13	6.25	3.13	3.13	3.13	12.5	12.5	3.13	>12.5	>12.5	>12.5	>12.5
	C17:1	12.5	>12.5	12.5	>12.5	3.13	6.25	6.25	6.25	3.13	6.25	>12.5	12.5	>12.5	>12.5	>12.5	>12.5	>12.5
CN (μg/mL)	C15:1	>12.5	>12.5	6.25	>12.5	12.5	>12.5	>12.5	12.5	12.5	>12.5	>12.5	>12.5	12.5	>12.5	>12.5	>12.5	>12.5
CD (μg/mL)	C15:1	>12.5	>12.5	3.13	>12.5	6.25	>12.5	12.5	12.5	12.5	12.5	12.5	>12.5	12.5	>12.5	>12.5	>12.5	>12.5

Fig. 6. Sensitivity of rumen representative bacterial species in pure culture to ginkgo (Kyuju) extract and individual alkylphenolic component.

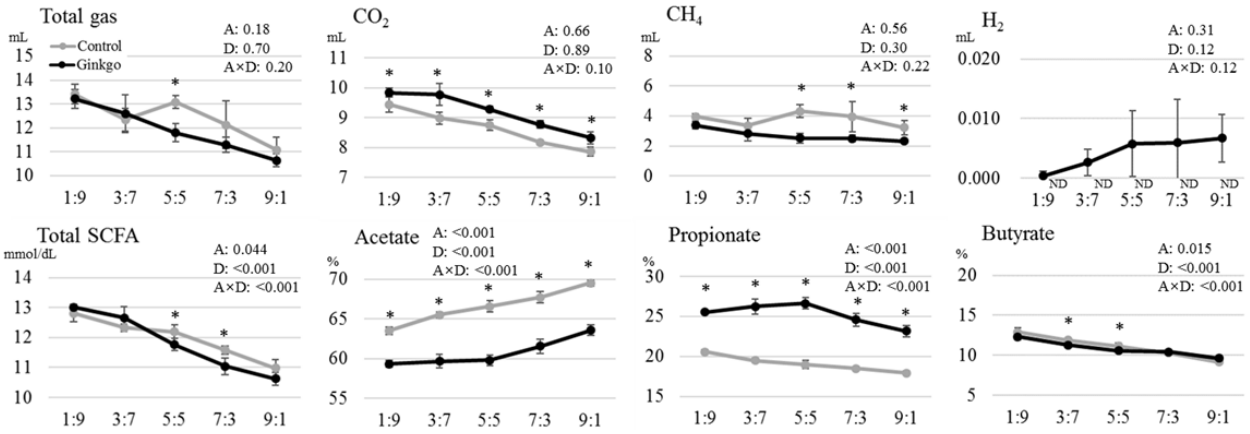

Fig. 7. Effect of ginkgo extract supplementation under different forage to concentrate ratios on gas and short chain fatty acid. Ginkgo extract (1.6% fruit equivalent in final culture) was added to treatment, while ethanol was to control. A: additive effect; D: diet effect; A×D: interaction of additive effect and diet effect. Asterisk (*) indicates significant difference between control and treatment under the same dietary condition (P<0.05).

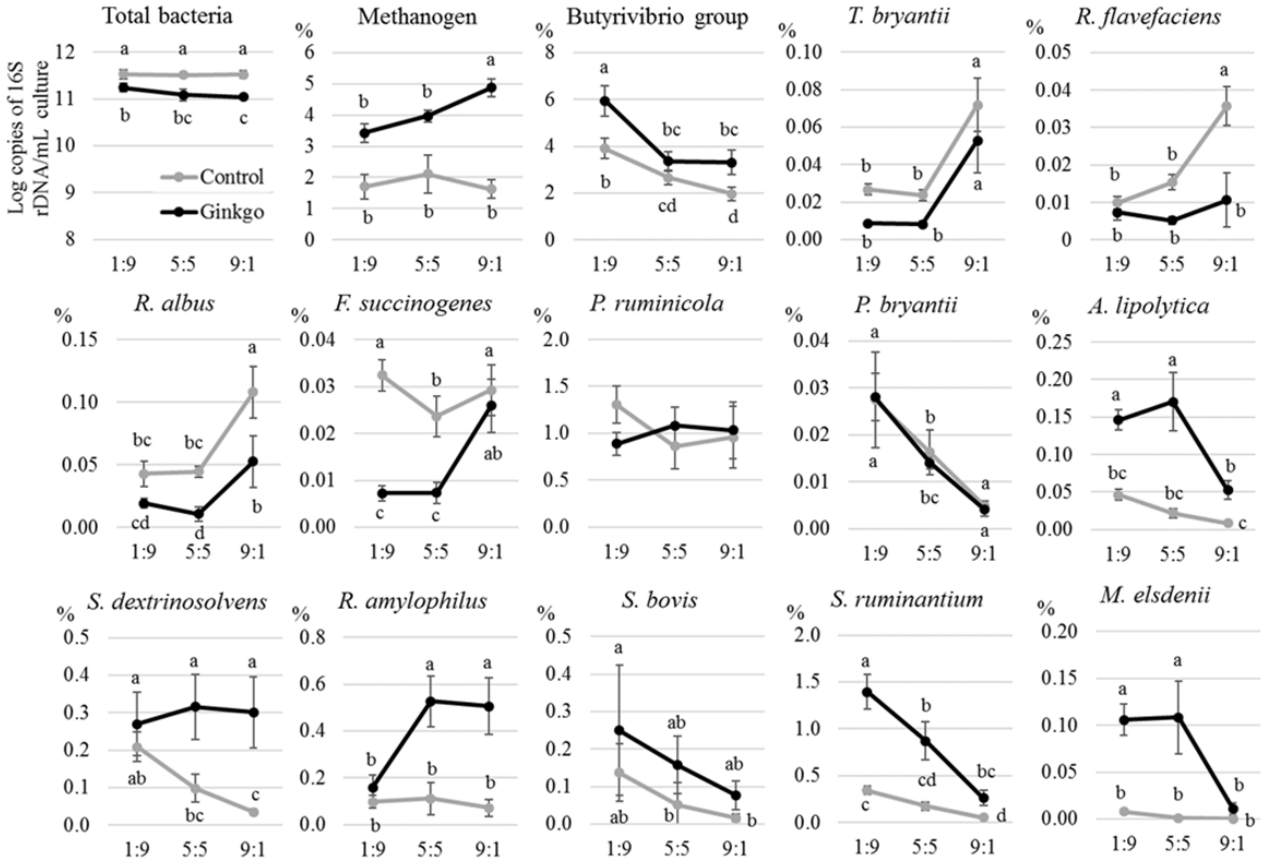

Fig. 8. Effect of ginkgo extract supplementation under different forage to concentrate ratios on rumen representative bacteria. Ginkgo extract (1.6% fruit equivalent in final culture) was added to treatment, while ethanol was to control. [a-d] Means with different superscripts differ (P<0.05).

affected by the F:C ratio, and decreased as the proportion of concentrate in the substrate decreased. Concentrations and proportions of acetate and propionate were affected by both ginkgo extract supplementation and diet; a decrease of acetate and increase of propionate were observed for the 5 F:C ratios tested.

The effect of ginkgo extract on bacterial levels under different F:C ratios is shown in Fig. 8 . Total bacteria was

decreased by ginkgo extract supplementation. The relative level of fibrolytic bacteria such as *R. flavefaciens, R. albus and F. succinogenes* was decreased by ginkgo extract, and the decrease was dependent on both the species and F:C ratio; the predominant *R. albus* showed obvious decreases at 5:5 and 9:1 ratios. Several species related to succinate and propionate production, such as *S. ruminantium, A. lipolytica, R. amylophilus, S. dextrinosolvens* and *M. elsdenii,* were increased with ginkgo extract under almost all dietary substrate conditions. These results suggest that rumen modulation by ginkgo extract can be achieved at a wide range of F:C ratios with no adverse impact on feed digestion. Moreover, F:C ratios of 5:5 and 7:3 may be optimal when methane mitigation is expected. The modulation of fermentation is clearly indicated to be a reflection of microbial changes by ginkgo extract.

Interpretation and perspective

Anacardic acid in ginkgo, accounting for 85% of alkylphenolic compounds (Fig. 1), was found to be a major compound to inhibit specific group of rumen bacteria. Although the alkyl side-chain structure is different (C13:0, C15:1 and C17:1), these anacardic acids in ginkgo were potent for selective inhibition of rumen bacteria (Fig. 6). Fruit of ginkgo contains a greater proportion of anacardic acids than other parts (leaves or seeds) that have been commercially used [12]. Therefore, the fruit is considered more functional as an antimicrobial and more easily available in terms of quantity than leaf and seed. It is known that other bioactive compounds besides alkylphenols in ginkgo extract such as flavonoid might influence rumen fermentation to decrease rumen methane [13]. Although more details on the mode of action of ginkgo extract is experimentally to be investigated, alkylphenols represented by anacardic acid could be a main factor in altering rumen microbiota and fermentation.

Ginkgo fruit extract altered rumen fermentation with no adverse effect on SCFA level, although extent of methane reduction was diet-dependent (Fig. 7). Hydrogen accumulation was observed only with ginkgo supplementation, indicating that hydrogen released from hydrogen-producing bacteria is not completely well metabolized via alternative pathways involved in propionate production rather than methanogenesis. SCFA composition was greatly changed by ginkgo extract toward less acetate and more propionate production. Such great shift in rumen fermentation pattern is attributed to rumen microbial changes by selective inhibition of rumen microbes with ginkgo supplementation (see below).

Ammonia reduction by ginkgo fruit extract in RUSITEC study could be related to inhibition of hyper-ammonia producing bacteria *C. stickandii* and *P. anaerobius* by ginkgo extract, i.e. these species were confirmed quite sensitive to ginkgo extract when those were individually cultivated (Fig. 6). If ammonia reduction by ginkgo extract successfully occurs within a critical level (>5mgN/dL), it might lead better dietary N economy for maintaining microbial protein synthesis [14]. The disappearance of DM, NDF, and ADF were not affected by ginkgo extract in RUSITEC study (Fig. 4). This is the most important characteristic to be addressed for developing a new additive candidate, as reported for CNSL [9]. However, longer incubation or feeding studies are obviously needed for more practical evaluation.

Rumen fermentation changes by ginkgo extract were reasonably attributed to microbial community changes that were evaluated MiSeq (Fig. 5) analysis, and also by sensitivity of individual rumen bacterial species to the extract (Fig. 6). Microbial selection by ginkgo extract was suggested by growth inhibition of hydrogen- and formate-producing bacteria (*R. flavefaciens* and *R. albus*) and by growth stimulation of succinate- and/or propionate-producing bacteria (*P. ruminicola*, *S. dextrinosolvens*, *R. amylophilus*, *S. ruminantium*, and *M. elsdenii*).

Although the abundance of fibrolytic organisms (*Fibrobacter*, *Ruminococcus*, fungi) was depressed by ginkgo extract supplementation, NDF and ADF digestibility was not affected (Fig. 4). This suggests development of an alternative fibrolytic system, possibly due to an increase in abundance of *Prevotella* and *Selenomonas* (Fig. 5), some groups of which are involved in fiber degradation [15, 16]. The abundance of *P. ruminicola* was not affected by F:C ratio, supporting that this bacterium might play an important role in digestion of a wide range of diet. The contribution of uncharacterized fibrolytic bacteria [17, 18] may also need to be considered for deeper discussion about fiber digestion.

In application, one problem needs to be solved: ginkgo fruit contains nutritionally harmful 4-methylpyridoxin (a vitamin B_6 antagonist)[19], which may be unsuitable for animal feed, leading to deficiency of vitamin B_6 for animals. If the activity of this anti-nutritional compound can be reduced by specific treatments, ginkgo fruit can be further evaluated in animal feed studies. One solution could be silage fermentation. Some lactic acid bacteria can produce vitamin B_6[20], which might compensate for the negative effects of 4-methylpyridoxin if those bacteria are used as a silage inoculant. This possibility needs to be confirmed to better assess the functionality of ginkgo fruit as a potential feed additive for ruminant animals.

Reference

1) Hook SE, Wright AG, McBride BW. 2010. Methanogens: methane producers of the rumen and mitigation strategies. Archaea, 2010: 945785.

2) Guan H, Wittenberg KM, Ominski KH, Krause DO. 2006. Efficacy of ionophores in cattle diets for mitigation of enteric methane. J. Anim. Sci., 84: 1896-1906.

3) McGuffey RK, Richardson LF, Wilkinson JID. 2001. Ionophores for dairy cattle: current status and future outlook. J. Dairy Sci., 84: E194-203.

4) Beauchemin KA, Kreuzer M, O'Mara F, McAlister TA. 2008. Nutritional management for enteric methane abatement: a review. Anim. Prod. Sci., 48: 21-27.

5) Cowan MM. 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12: 564-582.

6) Lewis K, Ausubel FM. 2006. Prospects for plant-derived antibacterials. Nat. Biotechnol., 24: 1504-1507.

7) Greathead H. 2003. Plants and plant extracts for improving animal productivity. Proc. Nutr. Soc., 62: 279-290.

8) Ministry of Agriculture, Forestry and Fisheries. Agricultural statistics. 2013. http://www.maff.go.jp/j/tokei/kouhyou/tokusan_kazyu/

9) Watanabe Y, Suzuki R, Koike S, Nagasima K, Mochizuki M, Forster RJ, Kobayashi Y. 2010. In vitro evaluation of cashew nut shell liquid as a methane-inhibiting and propionate-enhancing agent for ruminants. J. Dairy Sci., 93: 5258-5267.

10) Shinkai T, Enishi O, Mitsumori M, Higuchi K, Kobayashi Y, Takenaka A, Nagasima K, Mochizuki M, Kobayashi Y. 2012. Mitigation of methane production from cattle by feeding cashew nut shell liquid. J. Dairy Sci., 95: 5308-5316.

11) Czerkawski JW, Breckenridge G. 1977. Design and development of a long-term rumen simulation technique (Rusitec). Br. J. Nutr., 38: 371-384.

12) van Beek TA, Montoro P. 2009. Chemical analysis and quality control of *Ginkgo biloba* leaves, extracts, and phytopharmaceuticals. J. Chromatogr. A., 1216: 2002-2032.

13) Kim ET, Guan LL, Lee SJ, Lee SM, Lee SS, Lee ID, Lee SK, Lee SS. 2015. Effects of flavonoid-rich plant extracts on *in vitro* ruminal methanogenesis, microbial populations and fermentation characteristics. Asian-Australas. J. Anim. Sci., 28: 530-537.

14) Griswold KE, Apgar GA, Bouton J, Firkins JL. 2003. Effects of urea infusion and ruminal degradable protein concentration on microbial growth, digestibility, and fermentation in continuous culture. J. Anim. Sci., 81: 329-339.

15) Bekele AZ, Koike S, Kobayashi Y. 2010. Genetic diversity and diet specificity of ruminal *Prevotella* revealed by 16S rRNA gene-based analysis. FEMS Microbiol. Lett., 305: 49-57.

16) Sawanon S, Koike S, Kobayashi Y. 2011. Evidence for the possible involvement of *Selenomonas ruminantium* in rumen fiber diegstion. FEMS Microbiol. Lett., 325: 170-179.

17) Kobayashi Y. 2006. Inclusion of novel bacteria in rumen microbiology: Need for basic and applied science. Anim. Sci. J., 77: 375-385.

18) Jami E, Shabtay A, Nikbachat M, Yosef E, Miron J, Mizarhi I. 2012. Effects of adding a concentrated pomegranate-residue extract to the ration of lactating cows on in vivo digestivility and profile of rumen bacterial population. J. Dairy Sci., 95: 5996-6005.

19) Isah T. 2015. Rethinking *Ginkgo biloba* L.: Medicinal uses and conservation. Pharmacogn Rev., 9: 140-148.

20) Champagne CP, Tompkins TA, Buckley ND, Green-

Johnson JM. 2010. Effect of fermentation by pure and mixed cultures of *Streptococcus thermophilus* and *Lactobacillus helveticus* on isoflavone and B-vitamin content of a fermented soy beverage. Food Microbiol., 27: 968-972.

家 畜 栄 養 生 理 研 究 会 会 則

（総　　則）

第一条　本会は家畜栄養生理研究会と称する。

第二条　本会は家畜栄養生理に関する研究及びその成果の応用を促進する事を目的とする。

第三条　前条の目的を達成するため下記の事業を行う。

一．家畜栄養生理の研究に関する討議（春季・秋季集談会）

二．家畜栄養生理に関する研究情報、文献の蒐集配布並びに交換

三．家畜栄養生理に関する共同研究の促進及び研究相互の連絡

四．家畜栄養に関する研究成果及び技術につき必要と認めた場合、印刷物刊行等により普及を図ること

五．その他本会の目的達成のために必要な事業

（会　　則）

第四条　会員は正会員と賛助会員及び名誉会員とする。

第五条　本会の正会員になろうとするものは、会員の推薦により事務局に申し込むものとする。

２．事務局は前項の申し込みに対して可否を決定する。但し､名誉会員は評議員会が推薦し､総会において決定する。評議員は名誉会員に推戴された時点をもってその職を退任することとする。

３．会員は総会及び集談会に参加し発言することができる。但し、非会員も別途定める参加費を納入すれば、集談会に参加し、発言することができる。

４．名誉会員は評議員会に出席し、助言することができる。

第六条　会員は会費を納入するものとする。但し、名誉会員は会費を免除する。

２．正会員の年会費は4,000円とし、賛助会費の年会費は20,000円とする。年会費の徴収方法は事務局が決定する。

第七条　会員は下記の事項に該当する時は会員たる資格を失う。

一．本人の意志による退会

二．長期の会費未納の場合

三．会員として不適当と事務局が認め、総会がこれを承認した場合

但し、第一項により退会しようとする時は、事務局宛に届け出るものとする。

第八条　本会の事業年度は毎年3月１日に始まり翌年2月末日に終わる。

（役　　員）

第九条　本会に役員として会長1名、事務局員若干名、会計監査2名、評議員若干名、及び編集委員若干名を置く。役員の任期は2年とし、重任を妨げない。

第十条　役員は総会において選定する。

第十一条　会長及び事務局員は事務局を、編集委員は編集委員会を組織する。

２．事務局は総会で議決された方針に従って会務を運営する。

３．会長は本会を代表し、事務局員・編集委員の業務を統轄する。

４．事務局員は庶務、会計その他の業務を分担する。

５．編集委員は評議員の推薦による話題提供者の論文等を査読し、編集業務を担当する。

第十二条　評議員は本会の重要な事項につき会長の諮問に応じる。

（会　　議）

第十三条　会議は総会及び評議員会とする。

第十四条　総会を分けて通常総会及び臨時総会とする。

２．通常総会は毎年１回開催する。

３．総会は評議員会の議を得て会長がこれを召集する。

４．総会の議長はその都度選出するものとする。

第十五条　総会は本会の経理、人事及び事業の全般に亘る主要事項を審議決定する。

第十六条　評議員会は役員を以て構成する。

２．評議員会は会長がこれを召集し、その議長となる。

３．評議員会は原則として年2回開催する。

第十七条　評議員会は本会運営の基本方針を審議し、主要事項について総会へ提出し、その審議を求める。

（昭和59年4月6日改正）
（平成12年5月13日改正）
（平成15年5月10日改正）
（平成17年11月12日一部改正）
（平成27年11月14日一部改正）

栄養生理研究会報編集・投稿規程

1. 本会報は、家畜栄養生理に関する論文および研究会資料を掲載する。
2. 掲載論文の著作権は家畜栄養生理研究会に属する。
3. 評議員会は、春季集談会（自由課題形式）および秋季集談会（シンポジュウム形式）の話題を決定し、その話題提供者を推薦する。
4. 編集委員長より査読を委託された編集委員は、話題提供者等からの論文を査読し、著者に修正を求め、本研究会報への掲載可否を判断する。
5. 原稿はコンピュータソフト（MS-Word、一太郎など）を用いて作成し、ファイル（E-mail 添付ファイル、FD または CD-R）を事務局へ提出する。図表は本文とは別ファイルとして作成し、本文中には埋め込まない。図はモノトーンとする。電気泳動写真、顕微鏡写真などの原板は、鮮明なものを添付する。既報の図表を転載（加筆・修正も含む）する場合には、それらの出版元からの承諾書を添付する。図表を挿入する位置を本文左側の余白に朱書きで指示する。本文の句読点は“。”“、”を用いる。本文中に数値および英文を記載する場合は半角で入力し、コンマ(,)、セミコロン(;)、コロン（：)、ピリオド（.）を適当な位置に挿入する。
   イ）評議員より推薦を受け、本研究会報への原稿執筆を了承した話題提供者は、編集委員長が指定した期日までに原稿を事務局に提出する。
   ロ）原稿は原則として返却しない。
   ハ）原稿は簡易書留便で郵送する（FD または CD-R の場合）。
   二）原稿提出時に著者略歴（氏名、生年月日、学歴、職歴、専門分野）を添付する。
6. 原稿本文は原則として和文で書き、当用漢字を用いる。原稿はＡ 4 サイズの用紙を用い、上下左右とも 2.5 cm の余白を設け、35 字× 25 行の横書きとする。図表はＡ 4 紙 1 枚に 1 表、1 図を作成する。図の説明は、番号順に別紙にまとめて記載する。一論文の原稿枚数は、図表、図の説明を含めて 25 枚以内（約 1500 字が刷り上り 1 ページとなる）とする。本文には連続したページ番号を見やすい位置に記入する。行番号をページごとに付け、左側の余白に記入する。
   イ）原稿には表題、著者名、所属機関名を和文および英文で明記する。ただし英文は 1 ページ目の脚注に記す。
   ロ）特殊文字は用いず、ベタ打ちとし、原稿中には特殊文字などを赤字で明記する。
   ハ）引用文献は、本文中の関連箇所に肩つきで引用順に一連番号をつけ、番号はアラビア数字を用い片カッコで囲む。受理済みおよび印刷中のものを除き、投稿中の論文は引用文献として用いない。
   二）引用文献が雑誌の場合の書き方は、著者名、年号、表題、雑誌名、巻：頁一頁の順とする。
   （例）甫立京子・浜田龍夫・前田昭二 1995. 銅とビタミン E のアマニ油含有飼料への添加が子豚臓器中の銅，ビタミン E と過酸化脂質に与える影響．日畜会報 , 66：142-148.
   （例）Tomonaga S, Kaneko K, Kaji Y, Kido Y, Denbow DM, Furuse M. 2006. Dietary *β*-alanine enhances brain, but not muscle, carnosine and anserine concentrations in broilers. Anim. Sci. J., 77:79-86.
   ホ）単行本の記載は、著者名、発行年、書名、引用頁、発行所、発行地の順とする。
   分担執筆の場合は、所収の表題、編集または監修者名を加える。
   （例）糸川嘉則 1995. マグネシウム . 33-48. 光生館 . 東京 .
   （例）板橋久雄 2006. ルーメンにおける栄養素の代謝 . ルミノロジーの基礎と応用（小原嘉昭編），32-50．農文協．東京.
7. 初校は著者が行い、文章、図表の改変や追加は原則として認めない。校正の時に著しい改変や追加によって生じた費用は著者の負担とする。
8. 別刷りとして、筆頭著者に対し当該論文の pdf を無料配布する。
9. 栄養生理研究会報サイズはＡ 4 版とする。

（平成　7 年 10 月 21 日　評議員会審議）
（平成　8 年　4 月 20 日　評議員会承認）
（平成 11 年 10 月 30 日　評議員会承認）
（平成 13 年　5 月 19 日　評議員会承認）
（平成 15 年　5 月 10 日　評議員会承認）
（平成 18 年 10 月　7 日　評議員会承認）
（平成 19 年　3 月 28 日　評議員会承認）
（平成 19 年 11 月 30 日　評議員会承認）
（平成 25 年 11 月　9 日　評議員会承認）

家畜栄養生理研究会役員（平成 29 年度）名簿

（名誉会員）（16 名）

安保　佳一（元岩手大学農学部）
石橋　　晃（日本科学飼料協会）
板橋　久雄（日本科学飼料協会）
小原　嘉昭（明治飼糧水戸研究牧場）
加藤　和雄（東北大学大学院農学研究科）
川島　良治（元京都大学農学部）
木村　信熙（元日本獣医生命科学大学）
佐々木康之（元帯広畜産大学）
柴田　正貴（元畜産草地研究所）
新林　恒一（元家畜衛生試験場）
浜田　龍夫（元東京農業大学農学部）
左　　久（元帯広畜産大学）
藤原　　勉（元島根大学生物資源科学部）
本好　茂一（全国家畜畜産物衛生指導協会）
矢野　秀雄（元京都大学大学院農学研究科）
渡邉　泰邦（元信州大学農学部）

（評議員）（34 名）

秋葉　征夫（東北生活文化大学）
麻生　　久（東北大学大学院農学研究科）
新井　敏郎（日本獣医生命科学大学）
太田　能之（日本獣医生命科学大学）
大塚　　彰（鹿児島大学農学部）
奥村　純市（元名古屋大学農学部）
小堤　恭平（元畜産草地研究所）
小櫃　剛人（広島大学大学院生物圏科学研究科）
勝俣　昌也（麻布大学獣医学部）
喜多　一美（岩手大学農学部）
木田　克弥（帯広畜産大学）
久米　新一（京都大学大学院農学研究科）
黒瀬　陽平（北里大学獣医学部）
◎小林　泰男（北海道大学大学院農学研究院）
坂口　　英（岡山大学農学部）
佐々木晋一（元信州大学農学部）
佐藤　　幹（東京農工大学）
佐野　宏明（岩手大学農学部）
祐森　誠司（東京農業大学）
高橋　和昭（山形県立米沢女子短期大学）
竹中　昭雄（亜太糧食肥料技術中心）
寺田　文典（東北大学大学院農学研究科）
豊田　　淳（茨城大学農学部）
豊水　正昭（東北大学大学院農学研究科）
中川　二郎（豊橋飼料株式会社）
△古瀬　充宏（九州大学大学院農学研究院）
豊後　貴嗣（広島大学大学院生物圏科学研究科）
△松井　　徹（京都大学大学院農学研究科）
松本　光人（農林水産・食品産業技術振興協会）
村井　篤嗣（名古屋大学大学院生命農学研究科）
吉澤　史昭（宇都宮大学農学部）
米倉　真一（信州大学農学部）
盧　　尚建（東北大学大学院農学研究科）
和田　賢二（NOSAI 山形）

（編集委員）（10 名）

麻生　　久（東北大学大学院農学研究科）
小櫃　剛人（広島大学大学院生物圏科学研究科）
喜多　一美（岩手大学農学部）
黒瀬　陽平（北里大学獣医学部）
後藤　貴文（九州大学大学院農学研究院）
○小林　泰男（北海道大学大学院農学研究院）
辰巳　隆一（九州大学大学院農学研究院）
豊水　正昭（東北大学大学院農学研究科）
松井　　徹（京都大学大学院農学研究科）
盧　　尚建（東北大学大学院農学研究科）

◎会長　○編集委員長　△会計監事

賛　助　会　員

全国酪農業協同組合連合会購買部

豊橋飼料（株）テクニカルセンター

（五十音順）

栄　養　生　理　研　究　会　報
第61巻　第2号

平成29年9月発行（会員頒布：年会費4,000円）

発行者　小林　泰男

発行所　家畜栄養生理研究会
〒060-0809　札幌市北区北9条西9丁目
北海道大学大学院農学研究院動物機能栄養学研究室
家畜栄養生理研究会事務局
TEL & FAX: 011-706-2812
E-mail: eiyoseiri2011@gmail.com
郵便振替：口座番号01060-8-33558
加入者名：家畜栄養生理研究会

銀行口座：ゆうちょ銀行
店番818口座番号0207245
口座開設者名：家畜栄養生理研究会

印刷所　北海道大学生活協同組合　印刷・情報サービス部
〒060-0808　札幌市北区北8条西8丁目
北大クラーク会館内
TEL：011-747-8886
FAX：011-756-7971