ค่าความสมดุลของพลังงานและไนโตรเจนในขบวนการเมตาโบลิซึมของกระบือปลักที่เลี้ยงด้วยหญ้ารูซี่แห้งและเสริมกากถั่วเหลืองระดับต่าง ๆ

โตโมยูกิ คาวาชิมา[1] วิทยา สุมามาลย์ [2] พิมพาพร พลเสน [2]
รำไพร ใจเที่ยง [2] วัชรินทร์ บุญภักดี [3] ฟูมิโนริ เทราดะ [1]

บทคัดย่อ

การทดลองครั้งนี้เป็นการศึกษาถึงผลของระดับโปรตีนในอาหารของกระบือที่กินหญ้ารูซี่แห้งเสริมด้วยกากถั่วเหลืองระดับต่างๆ ที่มีต่อความสมดุลในขบวนการเมตาโบลิซึมของพลังงานและไนโตรเจน รวมถึงการย่อยได้ของเยื่อใยในกระบือ โดยใช้กระบือปลักจำนวน 4 ตัว เลี้ยงด้วยหญ้ารูซี่แห้งที่มีโปรตีนหยาบ 2.5% เป็นอาหารหยาบหลัก เสริมด้วยกากถั่วเหลืองเพื่อให้อาหารมีโปรตีนหยาบรวมแตกต่างกัน 4 ระดับ คือ 2.6%, 6.1%, 9.7% และ 13.3% จากการทดลองพบว่า ค่าการย่อยได้ของเยื่อใยหยาบ (CF) และเยื่อใย NDF ของหญ้ารูซี่แห้งในกระบือที่ได้รับหญ้ารูซี่แห้งเป็นอาหารอย่างเดียว ไม่แตกต่างกันทางสถิติกับกระบือที่มีการเสริมกากถั่วเหลืองทุกระดับ และพบว่ากระบือที่ได้รับอาหารที่มีโปรตีนหยาบสูง 13.3% มีการขับไนโตรเจนออกทางปัสสาวะมากกว่ากระบือที่ได้รับอาหารที่มีโปรตีนต่ำกว่าอย่างมีนัยสำคัญ ($p<0.05$) และมีอัตราส่วนของความร้อนที่ร่างกายผลิตขึ้นต่อพลังงานรวม (HP/GE) สูงกว่ากระบือที่ได้รับอาหารที่มีโปรตีนหยาบ 9.7% อย่างมีนัยสำคัญ ($p<0.05$) ถึงแม้ว่าอัตราส่วนนี้มีแนวโน้มลดลงตามระดับของโปรตีนหยาบที่เพิ่มขึ้นจาก 2.6%ถึง9.7%ก็ตาม กระบือปลักมีความสามารถในการย่อยอาหารเยื่อใยได้เป็นอย่างดีโดยไม่จำเป็นต้องเสริมโปรตีน นอกจากนั้นการเสริมอาหารที่มีโปรตีนหยาบรวมมากกว่า 10% ให้แก่กระบือ ไม่มีผลต่อการเพิ่มสมรรถภาพการเจริญเติบโตของกระบือ หรือบางทีอาจส่งผลเสียต่อสมรรถภาพการเจริญเติบโต หากกระบือได้รับแหล่งอาหารพลังงานที่ไม่เหมาะสมในด้านคุณภาพและปริมาณ

คำสำคัญ: กระบือปลัก, การย่อยได้ของเยื่อใย, การเสริมโปรตีน, ขบวนการเมตาโบลิซึมของพลังงาน, ขบวนการเมตาโบลิซึมของไนโตรเจน)

เอกสารวิชาการเลขที่ 44(3) – 0516(4) - 167

1 สถาบันปศุสัตว์และทุ่งหญ้าแห่งชาติ, ประเทศญี่ปุ่น

2 ศูนย์วิจัยและพัฒนาอาหารสัตว์ขอนแก่น, อ.เมือง, จ. ขอนแก่น 40260

3 กรมปศุสัตว์, พญาไท, กรุงเทพฯ 10400

Energy and nitrogen metabolism of swamp buffalo given Ruzi grass hay with different levels of soybean meal

Tomoyuki Kawashima [1] Witthaya Sumamal [2] Pimpaporn Pholsen [2], Rumphrai Chaithiang [2] Watcharin Boonpakdee [3] Fuminori Terada [1]

Abstract

A metabolism trail was conducted with four swamp buffaloes, given Ruzi grass hay with different levels of soybean meal, in order to examine the effects of protein level on energy and nitrogen balances as well as fiber digestion. The Ruzi hay contained only 2.5% of CP. The CP content in four dietary treatments ranged from 2.6%, 6.1%, 9.7% and 13.3% by supplement of soybean meal. The CF and NDF digestibility of Ruzi grass hay in the animals given only Ruzi grass hay were not different from those given the supplement of soybean meal. The nitrogen excretion into the urine of one animal given the ration containing 13.3% of CP was extremely high. A high CP content in the feed might have induced the animal to exhibit some metabolic disorder. The ratio of HP to GE was significantly higher in the animals given the highest amount of soybean meal (CP 13.3%) than in the animals given the ratio containing 9.7% of CP, although it decreased according to the level of CP up to 9.7%. Swamp buffalo has the ability to effectively digest fiber without protein supplement. On the other hand, protein supplement, which makes the total CP content more than 10%, would not be effective in improving the performance of swamp buffalo, or may be harmful to the animals depending on the quality and quantity of energy sources given.

(**Keywords**: Swamp buffalo, Fiber digestion, Protein supplement, Energy metabolism and Nitrogen metabolism)Technical document No. 44(3) – 0516(4) – 167

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1 National Institute of Animal and Grassland, Tsukuba, Ibaraki 305-0901, Japan

2 Khon Kaen Animal Nutrition Research and Development Center, Thapra, Muang, Khon Kaen,40260

3 Department of Livestock Development, Phayathai, Bangkok, 10400

Introduction

The number of swamp buffalo is now decreasing in Thailand and the nutrition of buffaloes is as neglected as the species itself. There is the general belief that buffaloes possess an inherent ability to utilize dietary fiber better than cattle. However, the manner in which the roughage is utilized need a lot more elucidation (Devendra, 1992). The digestibility of fiber and the utilization of energy tended to be higher in buffalo than in cattle when low quality roughage was given, although there was no significant difference (Kawashima *et al.*, 2000a). The present study showed some advantages of swamp buffalo in utilizing a low quality diet. Thus, a system to integrate swamp buffalo with crop production should be re-built to exploit agricultural by-products and to recycle nutrients back into the fields. This study is aimed at examining the effect of protein level on energy and nitrogen balances, and fiber digestion in swamp buffalo.

Materials and method

Four castrated male swamp buffalo (average body weight at the beginning of trial 441kg) were housed individually in metabolic crates with free access to water and subjected to the following four dietary treatments:

1) 100% of Ruzi grass hay (*Brachiaria ruziziensis*)
2) 92.1% of Ruzi grass hay and 7.9% of soybean meal
3) 84.3% of Ruzi grass hay and 15.7% of soybean meal
4) 76.4% of Ruzi grass hay and 23.6% of soybean meal

The treatments were conducted in this order. All animals were treated to remove endo- and ecto-parasites prior to the start of the experiment. Feed was offered in two equal meals at 0800 and 1700h and the daily amount given was 1.7% of animal's body weight. Each treatment consisted of a 9-day preliminary period and a 5-day collection period. Before starting the first treatment, a one-week additional preliminary period was assigned to every animal for the purpose of the adaptation to the new roughage and the metabolic crate. When any hay was refused during the collection period in treatment 1, the refusal was collected and subjected to chemical analysis. When the feed was refused during the preliminary period in treatment 2, 3 and 4, the amount was cut down so that the animals could consume all the feed. Mineral and vitamin premix with the following composition was given to each animal at 70 g per day: Vitamin A, 150,000 IU; Vitamin D_3, 30,000 IU; Vitamin E, 100 IU; Na, 11.81 g; Cl, 18.22 g; Ca, 330.00 g; P, 171.32 g; S, 1.03 g; Zn, 1.20 g; Fe, 499.30 mg; Mg, 6.03 g; Co, 15.10 mg; Cu 205.30 mg; I 15.30 mg; Mn, 499.50 mg; Se 7.00 mg; Mo 5.00 mg; K 4.70 mg; and filler (mixed all up to 1 kg total).

The amount of feces was measured over the five-day collection period. An aliquot of feces sample was dried at 60°C, left in a room, and measured as air dry matter. Five feces samples collected from each animal during the collection period were ground, mixed and subjected to chemical analysis. An aliquot of ground feces was dried at 120°C and measured as dry matter (DM). The total amount of urine was collected into acid and measured every day over the five-day collection period. After the last dietary treatment the animals were fasted for 4 days. Furthermore the total amount of urine excreted was collected over the last two days of the fasting period. Blood was collected from the jugular vein into a heparinized tube at 0730h, before feeding, at the end of feeding trial and at the end of fasting period. The plasma was separated by centrifugation and kept in a freezer until analysis was performed.

Oxygen consumption and the productions of carbon dioxide and methane were measured with a ventilated flow-through method, using a facemask, during the last 4 days of the feeding period and during the last 2 days of the fasting period. The system consisted of a face mask (Sanshin Kogyo Ltd., Japan), flow cell (Thermal flow cell FHW-N-S, Japan Flow Cell Ltd., Japan), oxygen analyzer (Xentra 4100, Servomex Ltd., UK), carbon dioxide and methane analyzers (Infra-red gas analyzer, VIA300, Horiba, Japan). Gas analyzers were calibrated against certified gases (Saisan Ltd., Japan), with known gas concentrations, at least twice a day. These measurements were conducted 6 times per days, each 6-10 minutes in duration, with the following schedule: 0700, 1000, 1300, 1600, 1900, 2200 and 0100h.

The DM, crude protein (CP), ether extract (EE), crude fiber (CF), and ash in oven dried (60°C) feed and feces samples were determined by the method of AOAC (1975). Acid detergent fiber (ADF) was determined by the method of

Goering and Van Soest (1970) and neutral detergent fiber (NDF) by the method of Van Soest *et al*. (1991). The nitrogen content in urine was determined by the method of AOAC (1975). Glucose and urea nitrogen (PUN) in blood plasma were measured using diagnostic kits (Biotech Reagent, Thailand) based on the enzyme-calorimetric method. The total protein content in the blood plasma was measured using diagnostic kits (Biotech Reagent, Thailand) based on the Biuret method. Non esterified fatty acid (NEFA) in the blood plasma was measured using diagnostic kits (NEFA C-Test Wako, Wako Pure Chemical Industries, Ltd.). Albumin in the blood plasma was measured using diagnostic kits (Biotech Reagent, Thailand) based on the bromocresol green method. Heat combustion of oven-dried feed and feces samples, and oven-dried (60^{o}C, 48 hours) urine were also determined using an adiabatic calorimeter (Shimadzu CA-4PJ, Japan). Heat of production (HP, kJ) was calculated by the equation, HP = $16.18*O_2 + 5.02*CO_2 – 2.17*CH_4 – 5.99*N$, where O_2, CO_2 and CH_4 represent volumes of oxygen consumed, carbon dioxide and methane produced (l) and N is the quantity of urinary nitrogen excreted (g) (Brouwer, 1965).

A general linear model (SAS, 1989) was used to analyze the effects of dietary treatments with a model including treatments and individual animals. Duncan's new multiple range test was applied to analyze the differences among the treatments.

Results

The chemical composition of feed and the ratio of the ingredients are shown in Tables 1 and 2, respectively. The CP contents of Ruzi grass hay and soybean meal were 2.5% and 48.1%, respectively. The CP contents of the feed ranged from 2.6% to 13.3% depending on the levels of soybean meal supplemented. As the animals refused feed in treatment 1 and 2, the DM intake was lower in treatment 1 and 2 than in treatment 3 and 4.

The digestibilities of nutrients are shown in Table 3. The DM and OM digestibilities were lower in treatment 1 than in the other treatments. The CF and NFE digestibilities showed similar trends as these of DM and OM, although these were not significant. The CP digestibility differed according to the levels of soybean meal supplemented. The digestibility of EE showed a similar trend as that of CP although the values in treatments 3 did not significantly differ from treatment 2 and 4. The ADF digestibility showed opposite trend to the other nutrients. That in treatment 1 was higher than in the other treatments.

The energy and nitrogen balances were compared among the treatments on the basis of metabolic body size (Table 4). Nitrogen excretion into urine of one animal in treatment 4 was extremely high (1.856 g/BWkg$^{0.75}$) in comparison with the average of the other animals in the same treatment (0.677 g/BWkg$^{0.75}$). Therefore, all the values of this animal in treatment 4 were eliminated in Table 4. The gross energy (GE), digestible energy (DE) and metabolizable energy (ME) intakes increased according to the levels of soybean meal. There was no difference in these values between treatments 3 and 4, although the animals in treatment 4 received more soybean meal, which had higher GE content. This was due to the increase in body weight, as the amount of feed given was calculated from the initial body weight. The energy loss into feces was the lowest in treatment 1, follow by treatment 2, and the highest in treatment 3. The value in treatment 4 did not significantly differ from treatments 2 and 3. Energy loss into urine was the highest in treatments 3 and followed by treatments 4, 2 and 1 in this order in the dietary treatment. The energy loss into urine during fasting was lower than treatment 1. Energy loss into methane was higher in treatments 2, 3 and 4 than in treatment 1. Heat production significantly increased according to the levels of soybean meal supplemented. The fasting heat production was not significantly different from that in treatment 1. Energy retention also increased according to the levels of soybean meal supplement, although there was no significant difference between treatment 3 and 4.

The ratios of DE to GE, and ME to GE, *i.e.* metabolizability were lower in treatment 1 than the other treatments. Those values in treatment 4 were the highest, although there was no difference among treatments 2, 3 and 4. The ratios of urine to GE, methane to GE, and ME to DE were not different among the treatments. The ratio of HP to GE was the highest in treatment 1 followed by treatments 2 and 4. It was the lowest in treatment 3.

Nitrogen intake, excretion into urine and retention significantly increased according to the levels of soybean meal supplemented. Although nitrogen excretion into feces also showed a similar trend, there was no difference between treatments 3 and 4. Nitrogen excretion into urine during fasting did not differ from treatments 3 and 4. Metabolic fecal nitrogen excretion was estimated to be 3.91 gN/kgDM by regression analysis of fecal nitrogen excretion per DM intake (gN/kgDM) against CP content in feed (%).

Methane production per day was significantly lower in treatment 1 than in the other treatments. However, methane production per OM intake was not different among the treatments.

The GE, DE and ME contents on the basis of DM were also compared among the treatments. As soybean meal included higher energy, all values tended to increase according to soybean meal supplemented. But there was no significant difference in the ME content among treatments 2, 3 and 4.

Table 5 shows the comparison of nutrient digestibilities and nutritive values of Ruzi grass hay among the treatments. The values of soybean meal were calculated by an extrapolation of data in treatments 1-4. Then the values of Ruzi grass hay in each treatment were calculated by using the values of soybean meal obtained. There was no difference in the nutrient digestibilities and nutritive values of Ruzi grass hay among the treatments except for the ADF digestibility, Which tended to decrease according to the levels of soybean meal supplemented, and the value in treatment 1 was significantly higher than the treatments 3 and 4.The NEFA, glucose, total protein, PUN and albumin concentrations in blood plasma are shown in Table 6. The NEFA in treatment 1 was higher than in treatments 3 and 4 among the dietary treatments. The NEFA during the fasting period was higher than the values of the dietary treatments. Glucose was higher in treatment 4 than in treatment 1. The values of other treatments were between treatments 1 and 4. The total protein tended to increase according to the levels of soybean meal supplemented and it was higher in treatment 4 than in treatments 1 and 2. Total protein during fasting was significantly higher than the values during feeding except for treatment 4. The PUN increased according to the levels of soybean meal, but there was no significant difference among treatments 2, 3 and 4.

The value during fasting was not different from the values of treatments 2, 3 and 4. The albumin was higher in treatments 4 and 5 (fasting) than in treatments 1 and 3.

Discussion

In developing countries (tropical), the animal must depend on locally available byproducts of agriculture and industry, which are often deficient in certain nutrients. In these countries there is a need to optimize production from the available resources by providing minimum amounts of the deficient nutrients (Leng, 1995). Swamp buffalo is one of popular native animals utilized in Southeast Asian countries, and there is a general belief that buffaloes posses an inherent ability to utilize dietary fiber better than cattle (Dervendra, 1992). The Ruzi hay used in this trial contained only 2.5% of CP, which is even lower than the value of rice straw. However, the CF and NDF digestibilities of Ruzi grass hay were not improved by the supplement of soybean meal. On the contrary, the ADF digestibility decreased according to the supplement. The reason for the decrease in ADF digestibility by the supplement is not clear. Homma (1994) reported that ammonia nitrogen in rumen fluid was higher in swamp buffalo than in Holstein cattle when both animals were given Thimothy hay *ad libitum*. He suggested that this be related to higher nitrogen content in saliva in swamp buffalo and to their ability to concentrate rumen fluid by a higher ability to absorb water from rumen. Kawashima *et al*. (2000b) also reported ammonia nitrogen in rumen fluid was higher in swamp buffalo than in Brahman cattle. This ability would favor rumen microbes to digest fiber fraction in rumen, even if the feed does not contain sufficient protein. Pradhan (1992) reviewed the studies on the specific differences in the digestibility of feed between cattle and buffalo and suggested that there were no significant differences and likewise the trend in nutrient digestibility in cattle and buffalo was also inconsistent. On the other hand, according to the review of Ranjahn (1992), a lot of work in India on the comparative utilization of feed by buffalo and Zebu cattle has indicated that buffalo utilize fiber better than cattle (apparent digestibility coefficients are about 5-8% higher). In the previous report (Kawashima *et al*., 2000c), the fiber digestibility of Ruzi grass hay (CP 3.5%) by Brahman cattle was improved by the supplement of soybean meal so that the total CP content became 6.8%. It is suggested, therefore, that swamp
buffalo has an ability to digest fiber better than cattle only in the diet, which contains a CP less than about 5%, but the difference would become smaller in the diet, which

contains CP above this level. This would be one of the key reasons that there were conflicting reports about the fiber digestion ability of buffaloes.

Moran (1983) indicated that swamp buffaloes have a total nitrogen balance either equal to or higher than that of Zebu cattle, and that this is generally the result of a higher nitrogen intake and/or a lower urinary nitrogen output, rather than a lower fecal nitrogen output. Dervendra (1992) supported these findings. In comparing this study with the previous study (Kawashima *et al*., 2000c) on Brahman cattle, the difference in nitrogen balance between swamp buffalo and Brahman cattle is not clear. The metabolic fecal nitrogen excretion estimated was higher in buffalo (39.1 Ng/DMkg) than in Brahman cattle (31.1 Ng/DMkg). Although it requires further trials to clarify the difference in nitrogen balance between swamp buffalo and Brahman cattle, it would be unlikely that swamp buffalo is far better at nitrogen utilization than cattle.

The nitrogen excretion into urine of one animal in treatment 4 was extremely high. The CP content in the ration (13.3 %) might have induced the animal to exhibit some metabolic disorder. The ratio of HP to GE was significant higher in treatment 4 than in treatment 3, although the ratio was decreased until the CP content went up to 9.7%. The protein supplement, which makes the total CP content more than 10%, would not be effective to improve the performance of swamp buffalo, or may be harmful to the animals depending on the quality and quantity of the energy sources given.

In the previous study (Kawashima *et al*., 2000a), NEFA content in blood during fasting became significantly higher in cattle than in buffalo. On the other hand, the PUN was significantly higher in buffaloes than in cattle during the fasting period. It was considered, therefore, that water buffalo had an ability to maintain a higher level of urea recycled better than cattle, which might be related to a higher ability to mobilized energy from body tissue protein during fasting and consequently caused a higher PUN content in water buffalo. On the other hand, cattle mobilized more from fat and consequently showed higher NEFA content during fasting. In the present study, the PUN content was generally higher in buffalo and the level during fasting was higher than in cattle, the same as in the previous report, although the change during fasting was not so dramatic in comparison with the previous value. The NEFA did not show a similar trend as before. In the previous study, animals were given feed at almost maintenance level. But the animals in this study, buffalo and cattle, were given almost twice the ME for maintenance before fasting. The difference in the levels of feeding before fasting was considered to make the results rather in consistent.

Methane is considered a 'greenhouse gas'. Methane production by cattle typically accounts for 5.5% to 6.5% of GE intake (Johnson and Ward, 1996). The value in the present study ranged from 3.7% to 4.5%, which was relatively lower than their value. Although it cannot be quantified, a lower methane production might have been partly caused by the difficulty of swamp buffalo in adapting to the face mask, as described in the previous report (Kawashima *et al*., 2000c). Liang *et al*. (1989) also reported that the HP value estimated by the mask tended to be lower than that estimated simultaneously by the chambers, although the difference was not significant. Crutzen *et al*. (1986) applied the value of 50-kg CH4 per buffalo, per year, in the estimates of methane yield from animals. The estimates of methane from buffaloes were mainly based on the study of Pandey (1981) and Krishna *et al*. (1978). Pandey (1981) reported that body weight and feed demand are higher than fir cattle, so those buffaloes require a gross energy intake of 85 MJ per animal per day. Kearl (1982) applied 125 kcal and 137 kcal of ME requirement for maintenance per metabolic body size in non-lactating and lactating buffaloes, respectively, to set a feeding standard for buffaloes. These values are relatively higher than the values for beef cattle and lactating cow in the Japanese Feeding Standard (Agriculture, Forestry and Fisheries Research Council Secretariat, 1995 and 1994, respectively). The ME requirement is higher in a roughage based diet than in a concentrate based diet. As buffaloes are generally fed with a roughage based diet, the Me requirement might be set higher than for cattle. However, Pradhan (1992) reported that the basal metabolic rate was lower in buffalo than in cattle. In the previous report (Kawashima *et al*., 2000a), the fasting heat production in swamp buffalo was lower than in Brahman cattle. More details will be discussed in the following reports.

Krishna *et al*. (1978) estimated higher CH_4 yields of 9% in Indian cattle fed on a slightly above maintenance diet and low quality feed. On the other hand, Kurihara *et al*. (1997) reported that methane production per unit DM intake increased with the rise in CP content of diets from 4% to 9% in cattle. In the present study, there was no significant difference in methane production per OM intake among the treatments. The assumption that the animals fed on low quality feed produce more methane, which was applied by Crutzen *et al*. (1986) for the estimation of methane production by ruminants, cannot be simply applied to swamp buffalo as to cattle.

The energy requirement is not higher in buffalo than cattle. The methane production was not improved by protein supplement. In light of these two points, the values of methane emission from buffalo by Crutzen *et al*. (1986) may be over estimated. But, as the data on methane production in buffaloes are still limited, further studies are still required.

The number of water buffalo is now decreasing in Thailand. The present study showed some advantages of swamp buffalo in utilizing a low quality diet. However, large amounts of agricultural byproducts, such as rice straw and sugarcane top, are burnt without proper usage. Low organic matter and nutrient content characterize the soil, especially in Northeast Thailand where the largest population of swamp buffaloes exists. Therefore, a system to integrate swamp buffalo with crop production should be rebuilt to recycle organic matter back to crop fields.

Conclusion

From a metabolism trail of swamp buffaloes, given Ruzi grass hay with different levels of soybean meal, in order to examine the effects of protein level on energy and nitrogen balances as well as fiber digestion. It can conclude that the CF and NDF digestibility of Ruzi grass hay in the animals given only Ruzi grass hay were not different from those given the supplement of soybean meal. The nitrogen excretion into the urine of one animal given the ration containing 13.3% of CP was extremely high. A high CP content in the feed might have induced the animal to exhibit some metabolic disorder. The ratio of HP to GE was significantly higher in the animals given the highest amount of soybean meal (CP 13.3%) than in the animals given the ratio containing 9.7% of CP, although it decreased according to the level of CP up to 9.7%.

Swamp buffalo has the ability to effectively digest fiber without protein supplement. On the other hand, protein supplement, which makes the total CP content more than 10%, would not be effective in improving the performance of swamp buffalo, or may be harmful to the animals depending on the quality and quantity of energy sources given.

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Table 1. Chemical composition of feed

	DM[1]	OM	CP	EE	NFE	CF	NDF	ADF	GE
	--------------------				%DM			--------------------	MJ/DMkg
Ruzi grass hay	92.1	95.1	2.5	1.0	55.6	36.0	74.7	46.3	17.7

Soybean meal	89.9	93.3	48.1	4.4	34.6	6.1	13.4	4.3	20.2

[1]: DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extracts; NFE, nitrogen free extracts; CF, crude fiber; NDF, neutral detergent fiber; ADF acid detergent fiber and GE, gross energy.

Table 2. Feed composition and DM intake

Treatment		1	2	3	4	SE[1]
Ingredients						
Ruzi grass hay	% of DM	100.0	92.1	84.3	76.4	0.0
Soybean meal	% of DM	0.0	7.9	15.7	23.6	0.0
CP content	%	2.60	6.11	9.70	13.29	0.03
DM intake	$g/BWkg^{0.75}$	49.1^{c}	65.4^{b}	79.3^{a}	78.1^{a}	1.2

[1] : SE, standard error; DM. Dry matter; CP, crude protein.

[a, b, c] : Means with different superscripts among treatments that significantly differ (P<0.05)

Table 3. Digestibility of nutrients of swamp buffalo given Ruzi grass hay with different levels of soybean meal (%)

Treatment	1	2	3	4	SE[1]
DM	50.1^{b}	54.7^{a}	54.5^{a}	57.3^{a}	1.2
OM	52.2^{b}	56.9^{a}	56.6^{a}	59.4^{a}	1.2
CP	1.6^{d}	53.9^{c}	67.8^{b}	76.3^{a}	2.0
EE	29.2^{c}	46.5^{b}	54.6^{ab}	59.8^{a}	3.7
NFE	55.5	57.5	56.8	58.0	1.1
CF	51.3	56.8	53.0	54.3	1.8
NDF	51.6	54.3	49.2	51.6	1.5
ADF	55.2^{a}	49.7^{b}	46.3^{b}	45.6^{b}	1.6

[1] : SE, standard error; DM. Dry matter; OM, organic matter; CP, crude protein; EE, ether extracts; NFE, nitrogen free extracts; CF, crude fiber; NDF, neutral detergent fiber and ADF acid detergent fiber.

[a, b, c, and d] : Means with different superscripts among treatments that significantly differ (P<0.05)

Table 4. Energy and nitrogen metabolisms in swamp buffalo

Treatment		1	2	3	4	5[2]	SE[1]
Body weight	kg	430	424	430	434	427	3
GE intake	$KJ/BWkg^{0.75}$	870 ^{c}	1172 ^{b}	1437 ^{a}	1426 ^{a}		25
DE intake	$KJ/BWkg^{0.75}$	409 ^{c}	619 ^{b}	762 ^{a}	816 ^{a}		15
ME intake	$KJ/BWkg^{0.75}$	354 ^{c}	539 ^{b}	659 ^{a}	722 ^{a}		15
Energy loss into							
Feces	$KJ/BWkg^{0.75}$	461 ^{c}	553 ^{b}	675 ^{a}	610 ^{ab}		23
Urine	$KJ/BWkg^{0.75}$	23 ^{d}	28 ^{c}	41 ^{a}	36 ^{b}	19 ^{e}	1
Methane	$KJ/BWkg^{0.75}$	32 ^{b}	53 ^{a}	63 ^{a}	58 ^{a}		3
Heat production	$KJ/BWkg^{0.75}$	295 ^{d}	357 ^{c}	396 ^{b}	437 ^{a}	279 ^{d}	6
Energy retention	$KJ/BWkg^{0.75}$	59 ^{c}	182 ^{b}	262 ^{a}	281 ^{a}	-279 ^{d}	19
DE/GE		0.469 ^{b}	0.528 ^{a}	0.530 ^{a}	0.576 ^{a}		0.014
ME/GE		0.405 ^{b}	0.460 ^{a}	0.458 ^{a}	0.510 ^{a}		0.014
Methane/GE		0.037	0.045	0.044	0.040		0.002
Urine/GE		0.027	0.024	0.028	0.026		0.001
Heat production/GE		0.340 ^{a}	0.305 ^{b}	0.276 ^{c}	0.308 ^{b}		0.005
ME/DE		0.861	0.869	0.864	0.885		0.007
Nitrogen intake	$g/BWkg^{0.75}$	0.204 ^{d}	0.640 ^{c}	1.232 ^{b}	1.657 ^{a}		0.015
Nitrogen loss into							
Feces	$g/BWkg^{0.75}$	0.200 ^{c}	0.295 ^{b}	0.396 ^{a}	0.394 ^{a}		0.012
Urine	$g/BWkg^{0.75}$	0.098 ^{c}	0.177 ^{c}	0.445 ^{b}	0.677 ^{a}	0.540 ^{ab}	0.058
Nitrogen retention	$g/BWkg^{0.75}$	-0.095 ^{d}	0.168 ^{c}	0.391 ^{b}	0.584 ^{a}	-0.540 ^{e}	0.062
Methane production	L/day	75.5 ^{b}	124.7 ^{a}	149.0 ^{a}	138.8 ^{a}		8.2
Methane/OM	L/OMkg	17.3	21.5	21.1	19.7		1.1
GE content	MJ/kg	17.70 ^{d}	17.92 ^{c}	18.11 ^{b}	18.31 ^{a}		0.01
DE content	MJ/kg	8.30 ^{c}	9.47 ^{b}	9.61 ^{ab}	10.55 ^{a}		0.25
ME content	MJ/kg	7.17 ^{b}	8.24 ^{a}	8.30 ^{a}	9.34 ^{a}		0.26

[1] : SE, standard error; GE, gross energy; DE, digestible energy; ME, metabolizable energy; OM, organic matter.

[2] : Treatment 5 means the levels during fasting period. Data are the least square means for four animals.

[a, b, c, d, and e] : Means with different superscripts among treatments that significantly differ (P<0.05)

Table 5. Digestibility of nutrient and nutritive value of Ruzi grass hay

Treatment		1	2	3	4	SE[1]
DM	%	50.1	52.7	50.1	50.9	1.3
OM	%	52.2	54.9	52.2	53.0	1.3
CP	%	1.6	-0.9	-2.2	1.1	2.9
EE	%	29.2	32.4	30.7	27.6	5.0
NFE	%	55.5	56.9	55.4	56.0	1.2
CF	%	51.3	5 6.4	52.1	52.9	1.8
NDF	%	51.6	54.8	50.0	53.1	1.5
ADF	%	55.2[a]	50.1[a b]	47.1[b]	46.9[b]	1.6
<u>DE</u>	MJ/kg	8.30	8.79	8.17	8.23	0.27
ME	MJ/kg	7.17	7.60	6.92	6.82	0.37
TDN	%	50.0	52.6	50.0	50.7	1.2

[1] : SE, standard error; DM. Dry matter; OM, organic matter; CP, crude protein; EE, ether extracts; NFE, nitrogen free extracts; CF, crude fiber; NDF, neutral detergent fiber and ADF acid detergent fiber; DE, digestible energy; ME, metabolizable energy; TDN, total digestible nutrients.

[a, b] : Means with different superscripts among treatments that significantly differ (P<0.05)

Table 6. NEFA, glucose, total protein, urea nitrogen and albumin concentration in blood plasma of swamp buffalo given Ruzi grass hay with different levels of soybean meal

Treatment		1	2	3	4	5[2]	SE[1]
NEFA	mEq/l	0.269[b]	0.211[b c]	0.181[c]	0.153[c]	0.759[a]	0.021
Glucose	mg/dl	68.8[b]	82.0[a b]	79.7[a b]	98.9[a]	97.7[a b]	8.2
Total protein	g/dl	5.80[c]	5.33[c]	6.13[b c]	6.69[a b]	7.15[a]	0.24
PUN	mg/dl	2.4[b]	16.9[a]	19.6[a]	22.5[a]	20.0[a]	1.7
Albumin	g/dl	3.38[b]	3.55[a b]	3.21[b]	3.93[a]	3.78[a]	0.15

[1] : SE, standard error; NEFA, non-esterified fatty acid; and PUN, plasma urea nitrogen.

[2] : Treatment 5 means the levels during fasting period.

[a, b, c, and d] : Means with different superscripts among treatments that significantly differ (P<0.05)