Studies on Utilization of Tamarind Kernel Powder Extract Residue as a Feed for Ruminants

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ABSTRACT

Tamarind (*Tamarindus indica*) is distributed throughout tropical and subtropical regions in over 50 countries. Tamarind kernel powder extract residue (TKPER) is a by-product derived from the tamarind kernel powder that is obtained during the extraction of polysaccharides, which is widely used in the food industry in Japan. This research conducted *in vitro*, *in situ* and *in vivo* experiments respectively in wethers for evaluating nutrition values of TKPER as a new feed for ruminants.

Two types of TKPER (TKPER I and II), dried under different rotating speeds, were used in *in vitro* experiment to examine the gas production and fermentation characteristics by comparing to dry heat soybean (SB), soybean meal (SBM) and dry soybean curd residue (SBCR). The contents of crude protein (CP), ether extract (EE), neutral detergent fiber exclusive of residual ash (aNDFom) and non-fiber carbohydrate (NFC) (%) in TKPER I and II were 41.4 and 42.0, 11.9 and 15.0, 1.4 and 0.5, 36.1 and 33.7, respectively. The results showed TKPERs had significantly lower gas production than the other test feeds at each observation time. The *in vitro* dry matter (DM) and CP digestibility (%) of TKPER I and II were 67.7 and 64.9, and 64.5 and 58.0, respectively,

significantly lower than those of SB and SBM.

In *in situ* experiment using nylon bag technique in wethers, the results demonstrated that *in situ* DM degradation parameters, rapidly degradable fraction (*a*), slowly degradable fraction (*b*) and the rate constant for disappearance of *b* fraction (*c*), respectively, of TKPER were similar to those of SB. The *in situ* CP degradation parameters (*a*, *b* and *c*) of TKPER were similar to those of SBM. *In situ* effective DM and CP degradability of TKPER were significantly higher than those of SBCR.

Furthermore, the digestibility, nitrogen balance and ruminal fermentation of TKPER were examined in *in vivo* experiment by comparing to SB, SBM and SBCR. Four wethers (51.6 ± 5.5 kg) were assigned in a 4×4 Latin square design feeding TKPER, SB, SBM and SBCR with ryegrass straw (R) at a ratio of 1:1 at 2% of body weight in DM on a daily basis. The estimated DM, CP and NFC digestibility and total digestible nutrients of TKPER were 58.7, 94.6, 84.7 and 93.8%, respectively. Wethers fed the TKPER diet had lower retention of nitrogen and ruminal ammonia nitrogen contents at 4 h after feeding than those fed the SBM diet, which had values similar to the SB or SBCR diet. The TKPER feeding had higher propionate and lower butyrate content, as well as lower acetate to propionate ratio in rumen fluid than SBM feeding at 4 h after feeding.

From this research it could be concluded that TKPER can be used as a high-protein source feed to be substituted for an expensive ingredient for growing livestock, or as a high-NFC nutrition energy source feed for lactating and fattening livestock. It is noted that due to the absence of fiber in TKPER, appropriate roughage source should be

chosen together with TKPER to meet the fiber requirement of ruminants.

Key words: By-product, Digestibility, Gas production, Nitrogen balance, Rumen fermentation, Tamarind kernel powder, Wethers

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LIST OF PUBLICATIONS

Chapters 2 and 3 are the peer reviewed version of the following article:

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CHAPTER 1

General introduction

1.1 Utilization of by-products as animal feeds in worldwide

By-products are widely used as animal feeds in developed and developing countries to aim at decreasing the feed costs of animal production. Preston *et al.* (2014) reported the feed composition table for 280 by-product feeds of cattle and sheep in order to use the by-products in a much more efficient way. In fact, in addition to reducing feed cost, utilization of by-products is beneficial to other aspects. Firstly, the by-product feeds could provide higher nutrition values for animals than the traditional feeds because the remaining components like starch, protein, fat and fiber in the by-products are more concentrated than in the raw materials. For example, the crude protein (CP) contents of by-products soybean meal, brewers' grain and cottonseed meal are higher than those of raw products soybean, barley and whole cottonseed, respectively. Secondly, selling by-products as animal feeds instead of disposing them as waste could bring a lot of profit to the company or even to the country. In the U.S., the ethanol and biodiesel industries generate significant amounts of by-products that add to the animal feed supply. The soy and ethanol industries cannot be viable if they couldn't sell the by-products: soybean meal and dried distillers grains (Kanpp, 2014). Taking India as an example of developing countries, the utilization of by-products efficiently has promoted the livestock industry accounting for more than 40% of total agricultural sector, which is increasing substantially in GDP of the country (Jayathilakan *et al.* 2012). In addition, efficient utilization of by-products has direct impact on environmental pollution of the country. Prandini *et al.* (2016) suggest that the inclusion of dry pasta by-products in the

diet for finishing heavy pigs could be an efficient feeding strategy to promote the recovery of wastes of the pasta industry so that it may avoid the pollution from discarding.

1.2 Utilization of by-products as animal feeds in Japan

In Japan, the food industry is a major part of the economy, accounting for 10% of total industrial production (Kajikawa, 1996), and a huge amount of industrial by-products are disposed each year. Livestock feeding in Japan relies heavily on imported feeds, which are easily affected by price fluctuation due to shortages. Utilization of by-products as feed ingredients in Japan is of great significance since it has improved self-sufficiency (Oishi *et al.* 2011). For instance, apple pomace (AP) is a by-product of apple juice production in Japan; the juice-making industry produces more than 20,000 tons of AP annually. The chemical composition of AP is characterized by a high content of moisture and sugar, together with a low content of CP. Hence, AP is particularly suitable for preparing total mixed ration (TMR) silage (Alibes *et al.* 1984; Gasa *et al.* 1992; Kennedy *et al.* 1999). Fang *et al.* (2016) evaluated the fermentation characteristics of TMR silages prepared with differing proportions of AP and their potential nutritive. The proportion of AP in TMR silages should be less than 5% of dietary DM. Take another two food waste as examples in Japan, soybean curd is a popular food and soy sauce is also a popular traditional seasoning. In the processing of soybean curd and soy

sauce, approximately 672,000 tonnes of soybean curd residue (SCR) and 86,000 tonnes of soy sauce cake (SSC) are produced annually in Japan (Kajikawa, 1996). However, most of the SCR and SSC is incinerated or buried in landfill. Yasuda *et al.* (2015) indicate that diets including SCR and SSC can be used as a substitute for a barley-and-corn-based concentrate for beef fattening.

1.3 Brief overview of tamarind

Tamarind (*Tamarindus indica*) is widely distributed throughout tropical and subtropical regions. Tamarind tree is a slow-growing, long-lived, and can reach a height of 24-30 m and may attain a spread of 12 m under favourable conditions. At present tamarind is cultivated in over 50 countries in the world. The major areas of production are: Cameroon, Central African Republic, Ethiopia, Guinea, Kenya, Nigeria, Senegal, Sudan, Tanzania, Uganda, Afghanistan, Australia, Bangladesh, Brazil, Cambodia, China, Colombia, Cuba, Egypt, Malaysia, Mexico, Myanmar, Nicaragua, Pakistan, Philippines, Sri Lanka, India and Thailand.

There are huge amount of products from tamarind in many food processing industries (Tsuda *et al.* 1994; Bhatta *et al.* 2001). Tamarind pulp is rich in pectin and contains significant amount of organic acids, 98% of which is tartaric acid. In India, the pulp is often an important ingredient for a variety of food products such as chutneys,

curries and sauces. Tamarind seed is a by-product of the commercial uses of tamarind fruit, which is rich in polysaccharides, proteins and lipids. Polysaccharides from tamarind seeds can be useful as a gel formation agent, and may be substituted for fruit pectins. The polysaccharide is composed of (1 → 4)-β-d-glucan backbone substituted with side chains of α-d-xylopyranose and β-d-galactopyranosyl (1 → 2)-α-d-xylopyranose linked (1 → 6) to glucose residues (Goyal *et al.* 2007). In particular, tamarind xyloglucan, also known as tamarind gum, is the major component of tamarind kernel powder, forms a stiff gel and is used for thickening, stabilizing and gelling in food. It is commercially available as a food additive for improving the viscosity and texture of processed foods.

1.4 Tamarind kernel powder extract residue

The tamarind kernel powder (TKP) which is made from the white tamarind kernel after being removed the black peel, is a crude extract of tamarind seeds. It has been used as a replacement for starch in cotton sizing, and as a wet-end additive in the paper industry, where it replaces starch and galactomannans (Glicksman, 1986). In Japan, TKP is imported for production of food additives. Firstly, TKP is impregnated with alcohol and alkali as pretreatment. Then purification is the key step during the whole production process. During this step, the polysaccharide is extracted from the TKP while proteins and lipids are remained in residue. The main product, polysaccharide

thickener, is now widely used in various food additive productions, such as tonkatsu sauce, dressing, mayonnaise and ice-cream. In the current situation, the waste was dealt with by the evaporation concentration method and then incinerated or buried in landfill. In fact, the waste, tamarind kernel powder extract residue (TKPER), could be considered as a by-product from the production line due to its high contents of protein and lipids.

1.5 Objectives of this research

To our knowledge, there have been no studies on the use of TKPER as a by-product feed in ruminant production. Therefore, the objectives of this research were as follows:

1. to determine *in vitro* gas production, digestibility and fermentation characteristics of TKPER, comparing soybean product and by-products (Chapter 2)
2. to determine the *in situ* ruminal degradation characteristics of TKPER in a comparison with soybean product and by-products (Chapter 3)
3. To evaluate *in vivo* digestibility, nitrogen balance and ruminal characteristics of feeding tamarind kernel powder extract residue, comparing soybean product and by-products (Chapter 4).

CHAPTER 2

In vitro ruminal fermentation of the tamarind kernel powder extract residue

2.1 Introduction

Tamarind (*Tamarindus indica*), which is widely distributed throughout tropical and subtropical regions, has been used as food materials in many food processing industries (Tsuda *et al.* 1994; Bhatta *et al.* 2001). Tamarind pulp is often an important ingredient for a variety of food products such as chutneys, curries and sauces. Tamarind seed is a by-product of the commercial uses of tamarind fruit, which is rich in polysaccharides, mostly galactoxyloglucan (Gidley *et al.* 1991), and proteins and lipids. In addition, the tamarind seed coat has high content of tannins about 56.2 g/kg in whole tamarind seed (Panigrahi *et al.* 1989).

The TKPER is a by-product derived from the TKP that is obtained during the extraction of polysaccharides thickener, which is used as a thickening, stabilizing and gelling agent in the food industry in Japan (Glicksman 1986; Gidley *et al*. 1991). In Japan, approx. 400 tons of TKPER are generated annually at a single major factory, but most of the TKPER is buried in landfills.

Our objectives in the present study were to determine the nutritive values and characteristics of TKPER using *in vitro* ruminal fermentation in a comparison with soybean product and by-products, i.e., dry heat soybean (SB), soybean meal (SBM) and dry soybean curd residue (SBCR).

2.2 Materials and methods

2.2.1 Preparation of test feeds samples

The TKP in the present study was processed from dehulled and ground tamarind seeds, and then treated by alkaline solution and refined to extract polysaccharides for food additives. The residue from extraction was concentrated and dried using a compact disc dryer (SCD-1301; Nishimura Works, Saga, Japan) to obtain the two types of TKPER (TKPER I and II). TKPER I was dried under 1.0 rpm and the TKPER II was dried under 1.35 rpm. These procedures were executed at a chemical company located in Osaka Prefecture, Japan.

Dried SB and SBM were purchased from a feed company located in Hyogo Prefecture, Japan, and SBCR was processed and dried at a food factory in Kyoto Prefecture, Japan. Five types of test feeds, i.e., TKPER I and II, SB, SBM and SBCR, were ground with Willey mills using a 2-mm sieve.

2.2.2 In vitro ruminal fermentation

The *in vitro* gas production and the *in vitro* dry matter (DM) and crude protein (CP) digestibility were determined according to the method of Tilley and Terry (1963) with slight modifications (Suzuki *et al*. 1995; Okano *et al*. 2009).

Three ruminal cannulated wethers with body weights (BWs) of 49.8 ± 2.1 kg were fed concentrate (35.4% rice bran, 54.0% rolled barley, 6.7% alfalfa meal, 3.4% soybean meal and 0.5% vitamin mineral premix) and ryegrass straw at the ratio 6:4 (CP 12.0% and total digestible nutrients [TDN] 67.0%) at 2% of BW in two equal portions at 09.00

and 17.00 hours, and had free access to water and mineral blocks for 2 weeks. The rumen fluid was collected via a ruminal cannula before the morning feedings and carried to the laboratory immediately. The rumen fluid was pooled among the wethers and filtered through four layers of cheesecloth prior to being combined with buffer. Samples (0.5 g DM) of TKPER I and II, SB, SBM and SBCR were each placed in 50-mL centrifuge tubes, and 40 mL mixed solution with rumen fluid and McDougall's artificial saliva at the ratio 1: 4 (McDougall 1948) were added and triplicated for each test feed. Each centrifuge tube, closed with a rubber stopper, was fitted with a 50-mL plastic syringe to measure gas production at the time intervals of 0, 3, 6, 9, 12, 15, 18, 21, 24 and 48 h. Each tube was then incubated in a water bath shaker at 39°C for 48 h. At the end of the incubation, the tubes were placed in ice water to terminate fermentation. Fermentation liquids and residues were separated by centrifugation (500 *g* for 5 min) for further analyses of fermentation characteristics and digestibility.

The cumulative gas production data were fitted to the non-linear model by Ørskov and McDonald (1979) using the Neway Excel Programme (Chen 1995) as Eq. 1:

$$Y = A + B\left(1 - \mathrm{e}^{-C(T-T_0)}\right) \qquad \text{(Eq. 1)}$$

where Y is the volume of gas (mL) produced at time (T), A is the gas production from the immediately soluble fraction (mL), B is the gas production from the insoluble fraction (mL), C is the gas production rate constant for insoluble fraction (mL/h), T is the incubation time (h), and T_0 is the start time of the gas production.

The wethers used in the *in vitro* experiment were managed according to the guidelines of the Kyoto University Animal Ethics Committee.

2.2.3 Chemical analyses

The test feeds used in the *in vitro* (TKPER I and II, SB, SBM and SBCR) experiment were analyzed for DM, CP, ether extract (EE) and crude ash (CA) according to the standards of the Association of Official Analytical Chemists (AOAC 2000; 930.15, 976.05, 920.39 and 942.05, respectively). Organic matter (OM) was calculated as weight loss through ashing. The values of neutral detergent fiber organic matter basis (aNDFom) and acid detergent fiber organic matter basis (ADFom) expressed exclusive of residual ash were analyzed as described by Van Soest *et al*. (1991).

The content of non-fiber carbohydrate (NFC) was calculated from CP, EE, aNDFom and CA by the following equation: NFC = 100 − (CP + EE + aNDFom + CA). Ruminal pH was measured using a glass electrode pH meter (Horiba, Kyoto, Japan). Ruminal NH_3-N was determined by the microdiffusion method (Conway 1962). Ruminal volatile fatty acid (VFA) concentrations were measured by gas chromatography (GC-14B; Shimadzu, Kyoto, Japan) equipped with a flame ionization detector (FID). The chromatography was carried out with a packed glass column (Thermon 3000-2% Shimalite TPA 60/80 3.2 mmφ × 2.1; Shimadzu). The temperatures of the column, detector, and injection part were 120°C, 250°C and 250°C, respectively, using nitrogen as the carrier gas.

2.2.4 Statistical analyses

We used the GLM procedure of Statistical Analysis System (SAS 1998) to analyze the *in vitro* data (i.e., the gas production at the observation times of 12, 24 and 48 h,

digestibility, the VFAs, NH_3-N and the gas production parameters). The model was $Y_{ij} = \mu + S_i + e_{ij,}$ where μ is the overall means, S_i is the fixed effect of the types of feed sample, and e_{ij} is the residual error. Significance was declared at $P < 0.05$.

2.3 Results

2.3.1 Chemical composition

The chemical compositions of the five test feeds are presented in Table 2.1. TKPER I and II had CP contents similar to that of SB, and the EE content of TKPER I was similar to that of SBCR. Very little aNDFom and no ADFom were found in both TKPER I and II. The NFC contents of TKPER I and II were higher than those of SB, SBM and SBCR.

Table 2.1 Chemical composition of test feeds (%)

Item	TKPER I	TKPER II	SB	SBM	SBCR
DM	93.5	92.6	92.3	90.4	93.5
OM*	90.8	91.2	94.2	93.2	95.2
CP*	41.4	42.0	42.7	49.0	25.8
EE*	11.9	15.0	18.8	1.5	10.8
aNDFom*	1.4	0.5	15.7	16.8	36.0
ADFom*	ND	ND	7.9	16.8	24.7
CA*	9.2	8.8	5.8	6.8	4.8
NFC[1]*	36.1	33.7	17.0	26.0	22.6

*on a DM basis; ND, not detected.

DM, dry matter; OM, organic matter; CP, crude protein; EE, Ether extract; aNDFom, neutral detergent fiber expressed exclusive of residual ash; ADFom, acid detergent fiber expressed exclusive of residual ash; CA, crude ash; NFC, non-fiber carbohydrate.

[1]calculated by 100 − (CP + EE + aNDFom + CA).

TKPER I and II, two types of tamarind kernel powder extract residue dried by compact disc dryer under 1.0 and 1.35 rpm, respectively; SB, dry heat soybean; SBM, soybean meal; SBCR, dry soybean curd residue.

2.3.2 In vitro gas production and digestibility

The *in vitro* gas production of both TKPER I and II were significantly lower than those of the other test feeds at the observation times of 12, 24 and 48 h ($P < 0.05$, Table 2.2). SBM showed the highest gas production at 12 h (38.1 mL/0.5g DM, $P < 0.05$) and SBCR had the highest gas production among the test feeds at 48 h (TKPER I, 45.1; TKPER II, 40.5; SB, 57.3; SBM, 82.9; and SBCR 100.8 mL/0.5g DM) ($P < 0.05$).

Both TKPER I and II were significantly lower in DM digestibility than the other test feeds ($P < 0.05$, Table 2.2). TKPER II had the lowest CP digestibility, and SB and SBM showed high CP digestibility ($P < 0.05$). No significant difference in CP digestibility was observed between TKPER I and SBCR.

Table 2.2 *In vitro* gas production and digestibility of test feeds

Test feeds	Gas production (ml/0.5g DM)			Digestibility (%)	
	12 h	24 h	48 h	DM	CP
TKPER I	18.8[a]	30.1[a]	45.1[b]	67.7[b]	64.9[b]
TKPER II	18.8[a]	28.9[a]	40.5[a]	64.5[a]	58.0[a]
SB	24.6[b]	47.6[b]	57.3[c]	79.7[c]	79.4[c]
SBM	38.1[c]	66.6[c]	82.9[d]	90.1[d]	82.9[c]
SBCR	27.6[b]	69.8[c]	100.8[e]	81.7[c]	67.5[b]
SEM	0.73	0.86	0.88	0.41	1.35

[abcde] Means in a column with different superscripts differ significantly ($P < 0.05$).

DM, dry matter; CP, crude protein; TKPER I and II, two types of tamarind kernel powder extract residue dried by compact disc dryer under 1.0 and 1.35 rpm, respectively; SB, dry heat soybean; SBM, soybean meal; SBCR, dry soybean curd residue; SEM, standard error of means.

2.3.3 In vitro gas production parameters

The parameters of *in vitro* gas production (*A*, *B* and *C* values) are shown in Table 2.3. The amounts of gas production from the immediately soluble fraction (*A*) of the five test feeds were all negative. The values of parameter *A* for TKPER I and II were significantly high ($P < 0.05$) and that for SBCR was the lowest ($P < 0.05$) (−3.8 and −5.7 vs. −23.4 mL). The gas production from the insoluble fraction (*B*) of SBCR was the highest at 152.7 mL ($P < 0.05$) and both TKPER I and II showed significantly lower *B* values than SB, SBM and SBCR ($P < 0.05$, respectively). The gas production rate (*C*) was not significantly different between TKPER I and SBCR, or between TKPER II and SBM. The T_0 values of TKPER I and II were not significantly different from that of SBM.

Table 2.3 *In vitro* gas production parameters[1] of test feeds

Test feeds	*A* (ml)	*B* (ml)	*C* (ml/h)	T_0 (h)
TKPER I	−3.8[c]	58.0[b]	0.038[a]	1.79[a]
TKPER II	−5.7[c]	48.9[a]	0.054[b]	2.32[ab]
SB	−13.7[b]	76.8[c]	0.060[c]	3.22[b]
SBM	−11.5[b]	103.4[d]	0.056[bc]	2.12[a]
SBCR	−23.4[a]	152.7[e]	0.038[a]	4.38[c]
SEM	1.15	1.87	0.001	0.207

[abcde] Means in a column with different superscripts differ significantly ($P < 0.05$).

[1]*A*, the gas production from the immediately soluble fraction (ml); *B*, the gas production from the insoluble fraction (ml);

C, the gas production rate constant for the insoluble fraction (ml/h); T_0, the start time of the gas production.

TKPER I and II, two types of tamarind kernel powder extract residue dried by compact disc dryer under 1.0 and 1.35 rpm, respectively; SB, dry heat soybean; SBM, soybean meal; SBCR, dry soybean curd residue; SEM, standard error of means.

2.3.4 In vitro ruminal fermentation characteristics

The pH of the ruminal fluid at collection was 6.66, and the pH of the *in vitro* fermentation liquid at the end of the 48-h incubation in the different test feeds ranged from 6.59 to 6.89 (Table 2.4). The total VFA contents of TKPER I and II were similar to those of SBM and SBCR but less than that of SB ($P < 0.05$, respectively). The ratios of acetate to propionate of TKPER I and II were similar to those of each of the other test feeds (Table 2.4). The NH_3-N content of both TKPER I and II were significantly higher than that of SBCR, and significantly lower than those SB and SBM ($P < 0.05$, respectively).

Table 2.4 *In vitro* ruminal fermentation characteristics of test feeds

Test feeds	pH	Total VFA (mmol/L)	Acetate (C2) (%)	Propionate (C3) (%)	Butyrate (nC4) (%)	C2 : C3	NH_3-N (mgN/dL)
TKPER I	6.89	85.3[a]	46.2	41.4[a]	12.5[c]	1.12[ab]	45.3[b]
TKPER II	6.86	95.8[a]	48.5	42.9[ab]	8.6[a]	1.14[ab]	42.0[b]
SB	6.84	157.4[b]	44.7	44.0[ab]	11.3[c]	1.02[ab]	59.4[c]
SBM	6.85	108.6[a]	44.9	45.8[b]	9.3[ab]	0.98[a]	69.5[d]
SBCR	6.59	115.6[ab]	49.1	40.0[a]	10.9[bc]	1.23[b]	24.3[a]
SEM	0.02	9.67	1.21	0.93	0.42	0.053	1.36

[abcd] Means in a column with different superscripts differ significantly ($P < 0.05$).

TKPER I and II, two types of tamarind kernel powder extract residue dried by compact disc dryer under 1.0 and 1.35 rpm, respectively; SB, dry heat soybean; SBM, soybean meal; SBCR, dry soybean curd residue; SEM, standard error of means.

2.4 Discussion

2.4.1 Chemical composition of TKPER

There was a small difference between the chemical compositions of TKPER I and II (Table 2.1) due to the different treatment conditions during the drying process. TKPER I was dried under lower rotating speed (1.0 rpm) and TKPER II was dried under higher rotating speed (1.35 rpm). The different rotating speeds of the dryer led to the different pressures for TKPER under drying treatment: TKPER I was dried under relatively lower pressure than TKPER II.

Since the tamarind kernel (original material) has no testa or husk and the TKPERs used in the present study were generated by purification treatment by alkaline solution, extremely low aNDFom and no ADFom contents were identified in both two types of TKPER. In contrast, TKPER I and II were rich in CP and EE. Moreover, TKPER I and II had high NFC contents, which might be due to insufficient extraction of polysaccharides from TKP.

2.4.2 In vitro characteristics of TKPER

The difference in gas production among the five test feeds in the *in vitro* experiment (SBCR > SBM > SB > TKPER I > TKPER II) was due to their difference in fiber contents (Tables 2.1 and 2.2). Cellulose in feed usually produces more gas than

starch, and starch produces more gas than protein (cellulose > starch > protein) (Fergus 2003). Hence, the very low aNDFom and ADFom contents of both TKPER I and II resulted in their lower gas production compared to the other test feeds. As for the *in vitro* gas production parameters, it is notable that the values of parameter *A* were negative for all of the test feeds (Table 2.3), indicating that there was a lag phase for all of the test feeds in the early stage of *in vitro* incubation.

Chumpawadee *et al*. (2007) reported that they obtained a negative value of parameter *A* in *in vitro* gas production for some feeds, i.e., rice bran, cassava chip and corn meal, and they explained the reason as the delay in microbial colonization of the substrate at early incubation. In the present experiments, among the five test feeds, the values of *A* (the immediately soluble fraction) of TKPER I and II were significantly higher than those of the other feeds ($P < 0.05$, Table 2.3), because tamarind kernel is rich in xyloglucan, a type of water-soluble polysaccharide (Goyal *et al*. 2007). The values of the start point of gas production (T_0) were lower for TKPER I and II than those for SB and SBCR, which also showed that there were faster happening of gas production for TKPER I and II, although the total gas productions of both TKPERs were lower (Table 2.2) due to their relatively lower fiber and higher NFC contents (Table 2.1). All five test feeds in the present study are concentrate feeds that are poor in structural carbohydrates; it was noted that samples with higher *in vitro* gas production usually consist mostly of structural carbohydrates (Blümmel *et al*. 1997). With fibrous and more slowly degraded feeds, the gas production rate increases (Menke & Steingass 1988; Lowman *et al*. 2002). The *B* values of TKPER I and II were significantly lower

than those of the other test feeds due to their low aNDFom and ADFom contents, which is consistent with the pattern of gas production (SBCR > SBM > SB > TKPER I > TKPER II, Table 2.2).

With respect to *in vitro* rumen fermentation, our present findings showed that the pH values of the five test feeds at 48 h of *in vitro* fermentation were 6.59-6.89, which is in the normal physiological range of rumen fluid for rumen microorganisms. The NH_3-N concentrations of the five test feeds ranged from 24.3 to 69.5 mgN/dL (Table 2.4), which is within normal rumen ammonia levels (<130 mgN/dL; Lloyd 1970) for ensuring microbial protein synthesis. The NH_3-N concentrations of the TKPERs were significantly higher than that of SBCR (Table 2.4). Miśta *et al.* (2014) mentioned that the low concentrations of ruminal NH_3-N of dried distillers' grains with solubles were due to their low concentrations of nonstructural carbohydrates. Therefore, the high NH_3-N concentrations of the TKPERs in the present study might be due not only to their high CP contents but also their high NFC contents. Although TKPER I and II had higher NFC contents, the VFA productions of TKPER I and II were lower than or similar to those of the other three test feeds (Table 2.4). This result might be due to the quite low aNDFom and ADFom contents of the TKPERs.

Regarding the *in vitro* DM and CP digestibility (Table 2.2), TKPER I and II had lower DM digestibility than SB, SBM and SBCR, and lower CP digestibility than SB and SBM, respectively. However, these results are not consistent with the results of our previous *in vivo* experiment (Wang *et al.* 2016), which showed no significant difference in DM or CP digestibility among TKPER I, SB and SBM. The discrepancy of the results

between that *in vivo* experiment and the present study may be caused by two reasons. The first possibility is that unlike the *in vivo* experiment, *in vitro* residues do not include metabolic fecal N in the assumption of digestibility (Ajmal Khan & Mahr-Un-Nisa Sarwar 2003). The *in vitro* indigestible residue may also contain bacterial residues and other substances, which would have been digested in the distal parts of the digestive tract *in vivo*.

There might be another reason for the difference in findings; the wethers in the previous study (Wang *et al*. 2016) were allowed adequate time to adapt to the test feeds. Here we investigated the delay of gas production for all five test feeds (Table 2.3), which might also reflect the problem of rumen fluid adaptation for the test feeds. Using rumen fluid from animals that were fed diets without test feed caused a prolonged lag period in an *in vitro* experiment (Hindle *et al.* 2004).

2.5 Conclusion

TKPER had good nutritive quality in light of its high contents of CP, EE and NFC, although it was not rich in aNDFom or ADFom. The low fiber contents in TKPER resulted in lower gas production at each observation time and lower *in vitro* gas production parameter *B* values compared to the other test feeds. However, neither TKPER I nor II showed any negative effect on the *in vitro* ruminal pH, VFA and NH_3-N contents. Although TKPER had lower *in vitro* gas production because of the low fiber content, we can conclude that TKPER could be used as a protein source feed.

CHAPTER 3

In situ ruminal degradation of tamarind kernel powder extract residue by nylon bag technique in wethers

3.1 Introduction

A large number of food processing wastes are generated in various food processing industries, and some of these wastes can be economically and environmentally valuable by-products as animal feeds when they are treated by appropriate preprocessing and/or feeding methods. Many experiments have been performed to assess the value of by-products from food processing as potential feed for ruminant production. For example, soybean curd residues can be an economically and nutritionally good feed ingredient for steers (Kim *et al*. 2012). Tomato pomace can be a very good-quality silage ensiling with wheat straw (Ziaei & Molaei 2010). The use of such by-products may not only improve the feeding value of poor-quality roughages but might also alleviate the environmental problems caused by the wastes (Pirmohammadi *et al.* 2006).

In Japan, a huge amount of by-products from the food industry need to be disposed of, and thus the use of these by-products as feed could be an effective way to both develop animal feeding systems and address the disposal problem. Several studies have reported the use of domestic by-products from food processing in Japan, such as brewer grain (Nishino *et al.* 2003), potato pulp silage (Okine *et al.* 2005), and polyphenol-rich winery wastes (Ishida *et al.* 2015), which are usually rich in energy, protein, fiber and/or some functional ingredient(s). As TKPER has a high protein content (Wang *et al.* 2016), it might be an alternative low-cost feed resource that could replace expensive high-protein feeds such as soybean product and by-products.

The objective of this study were to determine the *in situ* ruminal degradation

characteristics of TKPER by using nylon bag technique in a comparison with SB, SBM and SBCR.

3.2 Materials and methods

3.2.1 Preparation of test feeds samples

The TKP in the present study was processed from dehulled and ground tamarind seeds, and then treated by alkaline solution and refined to extract polysaccharides for food additives. Because TKPER I had higher gas production and digestibility than TKPER II in the *in vitro* experiment, the residue from extraction was concentrated and dried using a compact disc dryer (SCD-1301; Nishimura Works, Saga, Japan) to obtain the TKPER, which was dried under 1.0 rpm. These procedures were executed at a chemical company located in Osaka Prefecture, Japan.

Dried SB and SBM were purchased from a feed company located in Hyogo Prefecture, Japan, and SBCR was processed and dried at a food factory in Kyoto Prefecture, Japan. Four types of test feeds, i.e., TKPER, SB, SBM and SBCR, were ground with Willey mills using a 2-mm sieve.

3.2.2 In situ ruminal degradation

Four ruminal cannulated wethers with BWs of 55.6 ± 4.6 kg were used in a 4×4

Latin square design experiment for four periods. The wethers were housed individually in metabolic cages and fed concentrate and ryegrass straw at the ratio 6:4 (CP 12% and TDN 67.0%) at 2% of BW on a DM basis in two equal portions at 09.00 and 17.00 hours, and allowed to have free access to water and mineral blocks for 4 weeks. No refusal of the diet was observed throughout the experimental period.

Nylon bags (bag size 5×10 cm, pore size 50-55 μm, BG510, Bar Diamond, Parma, ID, USA) were prepared, and 3-g dry feed samples of TKPER, SB, SBM and SBCR were each put into a separate nylon bag. The mouth of the nylon bags was tied properly and anchored with a 30-cm non-elastic thread to the top of a cannula. The nylon bags were incubated in the rumen for 0, 2, 4, 8, 24 and 48 h, respectively. Upon removal from the rumen after incubation, the nylon bags were washed directly under running tap water until the fluid was cleaned, squeezed gently and dried to a constant weight at 60°C for 48 h. Zero time (0 h) disappearance values were obtained by washing pre-soaked un-incubated bags. The samples were kept for further analyses of the disappearance of DM and CP.

The relationship between the disappearance *(P)* of DM and CP from the nylon bags and the incubation time (*t*) was nonlinearly fitted to the non-linear model by Ørskov and McDonald (1979) using the Neway Excel Programme (Chen 1995) as Eq. 2:

$$P = a + b\,(1 - e^{-ct}) \qquad \text{(Eq. 2)}$$

where *a*, *b* and *c* are constants to estimate the rapidly soluble fraction, the degradable fraction and the degradation rate for *b*, respectively, and *t* is the incubation

time. The effective degradability (ED) of the DM and CP were calculated as Eq. 3:

$$\mathrm{ED} = a + b \times c \div (c + k) \quad \text{(Eq. 3)}$$

where k is the estimated fractional outflow rate of particles from the rumen assuming 0.02 and 0.05 h^{-1} (AFRC 1983); 0.02 is for the maintenance of ruminants whereas 0.05 is for the moderate production of ruminants. The parameters a, b and c are the same parameters as described above.

The wethers used in the *in situ* experiment were also managed according to the guidelines of the Kyoto University Animal Ethics Committee.

3.2.3 Chemical analyses

The test feeds used in the *in situ* (TKPER, SB, SBM and SBCR) experiment were analyzed for DM, CP, EE and CA according to the standards of the Association of Official Analytical Chemists (AOAC 2000; 930.15, 976.05, 920.39 and 942.05, respectively). OM was calculated as weight loss through ashing. aNDFom and ADFom expressed exclusive of residual ash were analyzed as described by Van Soest *et al*. (1991). The content of NFC was calculated from CP, EE, aNDFom and CA by the following equation: NFC = 100 − (CP + EE + aNDFom + CA).

3.2.4 Statistical analyses

We used the GLM procedure of Statistical Analysis System (SAS 1998) to analyze the *in situ* data (i.e., the DM and CP disappearance at each incubation time, the ED of

DM and CP, and the DM and CP degradation parameters). The model was $Y_{ijkl} = \mu + T_i + P_j + A_k + e_{ijkl}$, where μ is the overall mean, T_i is the fixed effect of treatment, P_j is the fixed effect of period, A_k is the random effect of animal, and e_{ijkl} is residual error. Significance was declared at $P < 0.05$.

3.3 Results

3.3.1 In situ DM and CP disappearance rate

The *in situ* DM and CP disappearance rates of the experimental test feeds at 0, 2, 4, 8, 24 and 48 h are presented in Table 3.1. TKPER showed the highest DM disappearance among the test feeds at 2-h incubation and had significantly higher DM disappearance than SBCR in the first 24 h of incubation ($P < 0.05$). SB had the significantly highest DM disappearance rate at 24 h ($P < 0.05$), but no significant difference was observed among TKPER, SB and SBM at 48 h. Until 4 h, TKPER had a significantly higher CP disappearance rate compared to SBCR ($P < 0.05$), and at 24 h, the CP disappearance of TKPER was higher than that of SBCR but lower than that of SB ($P < 0.05$, respectively). The CP disappearance of TKPER was very similar to that of SBM at all incubation times.

Table 3.1 *In situ* DM and CP disappearance of test feeds

Item and time	TKPER	SB	SBM	SBCR	SEM
DM disappearance					
0	6.0	6.9	6.0	3.9	0.99
2	40.6^{d}	24.4^{b}	31.7^{c}	8.55^{a}	1.29
4	47.2^{c}	42.3^{bc}	38.7^{b}	14.5^{a}	1.44
8	52.7^{b}	49.2^{b}	50.4^{b}	42.5^{a}	1.36
24	72.5^{b}	79.9^{c}	72.4^{b}	62.0^{a}	1.52
48	90.0^{ab}	93.0^{ab}	94.5^{b}	87.3^{a}	1.41
CP disappearance					
0	4.9	2.2	4.1	4.7	0.75
2	19.4^{bc}	17.4^{b}	21.4^{c}	6.3^{a}	0.72
4	24.5^{b}	37.7^{c}	27.5^{b}	12.2^{a}	1.01
8	36.5^{a}	45.8^{b}	38.6^{a}	34.7^{a}	1.19
24	60.7^{b}	77.6^{c}	66.8^{b}	49.4^{a}	2.04
48	89.8	92.6	93.4	84.6	1.92

^{abcd} Means in a column with different superscripts differ significantly ($P < 0.05$).

DM, dry matter; CP, crude protein; TKPER I, tamarind kernel powder extract residue dried by compact disc dryer under 1.0 rpm; SB, dry heat soybean; SBM, soybean meal; SBCR, dry soybean curd residue; SEM, standard error of means.

3.3.2 In situ DM and CP degradation parameters and effective degradability

Table 3.2 shows the *in situ* DM and CP degradation parameters (*a*, *b* and *c*), and the ED of the DM and CP. The lag phase was zero for each test feed in both the DM and CP degradations. Both the rapidly soluble fraction (*a*) and the degradable fraction (*b*) of the *in situ* DM degradability were not significantly different among TKPER, SB and SBM. SBCR had the lowest *a* value and the highest *b* value among the test feeds ($P < 0.05$). TKPER had a significantly higher rate of DM degradation (*c*) than both SBCR and SBM ($P < 0.05$). Regarding *in situ* CP degradability, TKPER had a significantly higher *a* value compared to those of SB and SBCR ($P < 0.05$, respectively).

The SBCR had the highest *b* value (130%, $P < 0.05$), which indicated that the CP

degradation of SBCR did not match the fitting curve very well in this study. No significant difference in *b* values was observed among TKPER, SB and SBM. The rate of CP degradation (*c*) of TKPER was significantly higher than that of SBCR, and significantly lower than that of SB ($P < 0.05$). The *in situ* ED values of the DM and CP of TKPER were 73.8% and 71.4%; 64.1% and 49.7% at the ruminal outflow rate (*k*) of 0.02 and 0.05 h^{-1}, respectively, which were significantly higher than those of SBCR ($P < 0.05$).

Table 3.2 *In situ* degradation parameters[1] and the effective degradability[2] (ED) of test feeds

Item	TKPER	SB	SBM	SBCR	SEM
Dry matter					
a (%)	13.7^{b}	10.1^{b}	13.4^{b}	2.3^{a}	1.47
b (%)	69.1^{a}	81.8^{a}	78.3^{a}	91.7^{b}	1.58
c (/h)	0.14^{b}	0.09^{ab}	0.08^{a}	0.05^{a}	0.011
Lag (/h)	0	0	0	0	0
ED 0.02 (%)	73.8^{b}	77.2^{b}	76.0^{b}	68.0^{a}	1.00
ED 0.05 (%)	64.1^{b}	63.1^{b}	61.5^{b}	48.6^{a}	0.93
Crude protein					
a (%)	9.9^{b}	4.8^{a}	9.5^{b}	4.6^{a}	0.71
b (%)	97.4^{a}	87.2^{a}	94.6^{a}	127.9^{b}	4.56
c (/h)	0.036^{b}	0.087^{c}	0.044^{b}	0.022^{a}	0.002
Lag (/h)	0	0	0	0	0
ED 0.02 (%)	71.4^{ab}	75.5^{b}	74.1^{ab}	68.5^{a}	1.33
ED 0.05 (%)	49.7^{b}	60.0^{c}	53.5^{b}	41.8^{a}	1.00

^{abcd} Means in a column with different superscripts differ significantly ($P < 0.05$).

[1] *a*, *b* and *c* are constants to estimate the rapidly soluble fraction, the degradable fraction and the degradation rate for *b*, respectively.

[2] The effective degradability at ruminal outflow rates (*k*) of 0.02 and 0.05 h^{-1}.

TKPER I, tamarind kernel powder extract residue dried by compact disc dryer under 1.0 rpm; SB, dry heat soybean; SBM, soybean meal; SBCR, dry soybean curd residue; SEM, standard error of means.

3.4 Discussion

The *in situ* DM disappearance (Table 3.1) of TKPER was significantly higher than those of SBCR (2-24 h), SBM (2-4 h) and SB (2 h) due to the low fiber and high NFC contents of TKPER, because aNDFom and ADFom are negatively correlated with DM disappearance (Abdulrazak *et al*. 2000). The *in situ* DM degradability parameters of TKPER were similar to those of SB in the present study (Table 3.2), which were reflected by the DM disappearance of TKPER and SB (Table 3.1). In a study of the *in situ* degradability of grains and by-product feeds (Batajoo & Shaver 1998), the DM rapidly disappearing fraction and slowly disappearing fraction of brewers dried grains and barley were 14.5% and 57.2%, 19.2% and 69.9%, respectively, which are similar to the TKPER values of the *in situ* DM parameters *a* and *b* in the present study (Table 3.2).

The ED of the DM of TKPER as well as those of the other test feeds at 0.05 h^{-1} was lower than at 0.02 h^{-1} (Table 3.2), because 0.05 h^{-1} is usually used for the production of ruminants (such as milking and fattening), whereas 0.02 h^{-1} is used for the maintenance of ruminants. Similarly, Ørskov *et al.* (1980) reported that the effective degradability in the rumen decreased with the increase in the ruminal outflow rate. The ED of the DM of SBCR in the present study was the lowest among the four test feeds, which is in agreement with the study by Jeon *et al.* (2013); the ED values of the DM of SBCR were reported as 69.56% and 44.06% at the outflow rates 0.02 and 0.05/h, respectively.

The CP disappearance of TKPER was highly consistent with that of SBM at each time point (0, 2, 4, 8, 24 and 48 h), and the *in situ* CP degradation parameters of TKPER were not significantly different from those of SBM (Table 3.1 and 3.2), which indicates that the pattern of CP degradation in TKPER might be similar to that of SBM. The raw materials of SBM and TKPER are soybean and tamarind, respectively, both of which are leguminous plants (Bhattacharya *et al.* 1994), and their production processes are similar. Consequently, the CP content of TKPER was similar to that of SBM (Table 2.1), which may have led to the similar performance in *in situ* CP degradability (Tables 3.1 and 3.2).

TKPER had *in situ* CP degradation parameters *a* and *b* (Table 3.2) that are similar to those of brewers dried grains and dried shelled corn (*a* and *b*: 9.4% and 87.1%, 9.6% and 87.7%, respectively) (Batajoo & Shaver 1998). The ED values of the CP of TKPER were 71.4% and 49.7% at the ruminal outflow rates of 0.02 and 0.05 h^{-1}, respectively. In our *in vivo* experiment (Wang *et al.* 2016), the total tract digestibility of the CP in TKPER was 94.6%, which is higher than the *in situ* ED of the CP of TKPER. The difference might be due to the digestion of ruminal undegradable protein of TKPER in the lower digestive tract.

3.5 Conclusion

The *in situ* CP degradation parameters of TKPER were not significantly different from those of SBM, which indicates that the pattern of CP degradation in TKPER might

be similar to that of SBM. The results indicated that TKPER could be used as a protein source feed as a substitute for the expensive ingredient SBM in animal production.

CHAPTER 4

Effect of feeding tamarind kernel powder extract residue on *in vivo* digestibility, nitrogen availability and ruminal fermentation in wethers

4.1 Introduction

Utilization of by-products is of great importance in rearing of livestock, both in developing and developed countries. In India, for instance, various by-products such as rubber seed cake, mango seed kernel, tea waste and tamarind seed have been recommended for use at the 10-30% level in the concentrate of livestock rations (Punj, 1982). Taking Japan as an example of developed countries, the food industry is a major part of the economy, accounting for 10% of total industrial production (Kajikawa, 1996), and a huge amount of industrial by-products are disposed each year. Livestock feeding in Japan relies heavily on imported feeds, which are easily affected by price fluctuation due to shortages. Utilization of by-products as feed ingredients has been promoted in order to improve self-sufficiency (Oishi *et al.* 2011).

The nutritional characteristics of several food industrial by-products have been investigated in comparison to conventional feeds. In Japan, studies have examined the mixing and ensiling of these by-products with other feed ingredients. Ishida *et al.* (2012a) reported that TMR silage, including soy sauce cake and noodle waste that was well preserved with high fermentation quality showed significantly higher digestibility of DM and OM compared to barley- and corn-based concentrate. As for fattening of Japanese Black heifers is concern, no significant difference in DM intake or daily gain were observed between TMR silage feeding and the concentrate feeding (Ishida et al., 2012b). Yani *et al.* (2012) demonstrated that intake and adequacy of TDN were significantly higher in dairy cows fed the TMR silage than those fed commercial

concentrate-based TMR silage. Later, Yani *et al.* (2015) studied the effects of utilization of local food by-products as TMR silage materials in sheep. The TMR silage including potato waste, noodle waste and soybean curd residue had significantly higher DM and aNDFom digestibility and TDN content than control feeding which contained commercial concentrate in sheep.

The TKPER is a by-product of the processing of polysaccharide thickener. Numerically 400 tons of TKPER are produced annually in one of the major factories in Japan and most of them are incinerated or buried in landfill in spite of its high CP and energy contents. It's well-known that protein is a quite important resource of nitrogen for microorganism to synthesize microbial protein in the rumen synchronizing with utilization of other nutrients in the diets, such as fermentable carbohydrate. Oh *et al.* (2008) reported that rumen degraded protein increased by higher level and degradability of dietary protein, may increase release of free amino acids, peptides and soluble proteins in the rumen of Hanwoo steers. In rearing of dairy cows, protein in diet is a key factor which could insure an adequate supply of metabolizable protein and essential amino acids to allow maximal production of milk and milk protein. Milk production increased 0.75 kg/d when dietary CP was increased from 15 to 16% and 0.35 kg/d when CP was increased from 19 to 20% (NRC, 2001). Moreover, TKPER has advantages not only in protein content but also in price, which will cost less than several commercial concentrate. Hence, it might be possible to use TKPER as an alternative to certain conventional ingredients of commercial concentrate, such as soybean products and by-products.

The objective of this study was to examine *in vivo* digestibility, nitrogen balance and ruminal fermentation of TKPER compared to soybean products and by-products (SB, SBM and SBCR) in wethers.

4.2 Materials and methods

4.2.1 Preparation of feeds

The TKPER was prepared by a chemical company in the Osaka Prefecture, Japan. Briefly, TKP was processed from dehulled and ground tamarind seeds, and then treated with alkaline solution and refined to extract polysaccharide for food additives. The residue from extraction was concentrated and dried to get TKPER. The SB and SBM were purchased from a feed company located in Hyogo Prefecture, Japan. The SBCR was processed and dried in a food factory in Kyoto Prefecture, Japan. The chemical composition of feeds was analyzed as shown in Table 4.1. TKPER had similar CP and EE contents to SB on a DM basis. No aNDFom content was detected in TKPER. The NFC content of TKPER was higher than that of SB, SBCR and SBM.

Table 4.1 Chemical composition (%) of the test feeds and ryegrass straw

Item	TKPER	SB	SBM	SBCR	Ryegrass straw
Dry matter	88.5	93.4	89.8	92.6	89.2
Organic matter†	91.6	94.3	93.3	96.5	95.8
Crude protein†	42.4	39.7	51.5	27.4	5.8
Ether extract†	15.0	20.6	1.9	9.4	2.1
aNDFom †	ND	16.9	10.8	28.1	65.0
Crude ash†	8.4	5.7	6.7	3.5	4.2
NFC‡	34.3	17.1	29.1	31.6	22.9

† On a dry matter basis. ‡calculated by 100 - (CP + EE + aNDFom + CA). ND, not detected. aNDFom, neutral detergent fiber exclusive of residual ash; NFC, nonfibrous carbohydrate. TKPER, tamarind kernel powder extract residue; SB, dry heat soybean; SBM, soybean meal; SBCR, dry soybean curd residue.

4.2.2 Animals, treatments and experimental design

Four ruminal cannulated wethers with initial body weight (BW) of 51.6 ± 5.5 kg were used in a 4 × 4 Latin square design experiment. The wethers were housed individually in four metabolic cages at the animal shelter at Kyoto University, Kyoto, Japan. Each experimental period was 14 days, consisting of a 9-day adaptation period and a 5-day sample collection period. The wethers used in the experiment were managed according to the guidelines of the Kyoto University Animal Ethics Committee.

The experiment consisted of four treatments which were TKPER, SB, SBM and SBCR fed with ryegrass straw (R) at a ratio of 1:1; these are referred to as the TKPER-R, SB-R, SBM-R and SBCR-R treatments, respectively. The wethers were fed with the experimental diets at 2% of BW on a DM basis in two equal portions daily, at 08:30 h

and 17:30 h. They were allowed free access to water and mineral blocks. No refusal was observed throughout the experimental period.

All feces and urine were collected every morning before feeding during the 5-day sample collection period. The feces samples were collected manually, weighed, and then dried in an oven at 60°C for 48 h, after which the DM was weighed. The 5 days' collected feces from each wether were mixed together, and 50 g of the mixed samples were ground with a Willey mill to pass through a 1-mm screen, and then stored for further analyses. The digestibility and TDN contents of the test feeds were calculated by subtracting the digestibility and TDN contents of ryegrass straw from those of total experimental diets, according to the method described by the National Agriculture and Food Research Organization (NARO, 2010).

The digestibility of a nutrient in the test feed being fed in form of mixed feed is calculated as follow: Digestibility of nutrient in test feed (%) = (A − B × C) × 100% / D, A: Digestibility of nutrient in total diet; B: Digestibility of nutrient in basal diet (ryegrass straw); C: proportion of total nutrient in diet supplied by basal diet; D: proportion of total nutrient in diet supplied by test feed.

The TDN content of each test feed was estimated by the following equation: TDN content = digestible OM content + digestible EE content × 1.25 (NARO, 2010). The urine samples were collected in plastic trays, 45 mL sulfuric acid (20%) was added, and the volume was measured. The 5 days' collected urine samples from each wether were mixed together, and 50 mL of the mixed samples were put into a plastic bottle and stored at -20°C for further analyses.

The rumen fluid samples were collected at 0 h (before feeding) and 4 h after feeding on the morning of the last day of each experimental period. The rumen fluid was filtered through four layers of gauze, and then analyzed for pH immediately using a glass electrode pH meter (Horiba Ltd., Kyoto, Japan). It was then centrifuged at 500 × *g* for 5 min and the supernatants were stored at -20°C for further analyses.

Jugular blood samples of approximately 10 mL were collected one time from each wether in vacuum plasma tubes at 0 h (before feeding) on the last day of each experimental period. The collected blood samples were centrifuged at 2600 × *g* for 15 min, and the plasma was stored at -20°C for further analyses.

4.2.3 Chemical analyses

The four test feeds, TKPER, SB, SBM and SBCR, and ryegrass straw were sampled from 4 batches during the metabolic experiment, and analyzed for DM, CP, EE and CA according to the standards of the Association of Official Analytical Chemists (AOAC, 2000). The content of OM was calculated as weight loss through ashing. aNDFom was analyzed according to the procedure described in Van Soest et al (1991). The content of NFC was calculated from CP, EE, aNDFom and CA using the following equation: NFC = 100 − (CP + EE + aNDFom + CA).

The feces samples were analyzed for DM, CP, EE, OM, aNDFom and NFC, as described above. Urine samples were analyzed for nitrogen (N) content using the Kjeldahl procedure. Ruminal ammonia nitrogen (NH_3-N) was determined by the

microdiffusion method (Conway, 1962). VFA concentrations were measured by gas chromatography (GC-14B; Shimadzu, Kyoto, Japan) equipped with a flame ionization detector (FID). The chromatography was carried out with a packed glass column (Thermon 3000-2% Shimalite TPA 60/80 3.2 mmφ × 2.1; Shimadzu). The temperatures of the column, detector, and injection were 120, 250 and 250°C, respectively, using nitrogen as the carrier gas.

The blood plasma samples were analyzed for glucose (Glu), total protein (TP), albumin (Alb), blood urea nitrogen (BUN), nonesterified fatty acid (NEFA), total cholesterol (T-Cho), phospholipids (PL), calcium (Ca) and inorganic phosphorus (IP) using diagnostic kits (Glucose-HR II, NEFA-HR, Albumin-HR II, L type Wako UN, L type Wako CHO·H, L type Wako Phospholipids, CalciumE-HA and Inorganic phosphorus-HR II, Wako Pure Chemical Industries, Ltd., Tokyo, Japan). Aspartate aminotransferase (AST), alanine transaminase (ALT) and γ-glutamyl transpeptidase (γ-GTP) activities were analyzed according to the standard methods established by the Japan Society of Clinical Chemistry (Kotani *et al.* 1994).

4.2.4 Statistical analyses

Data on chemical composition of diets, intake, apparent digestibility, nitrogen balance, ruminal pH, ruminal NH_3-N and blood metabolites were analyzed using general linear model (GLM) procedure of the Statistical Analysis System (SAS, 1998). The model was $Y_{ijkl} = \mu + T_i + P_j + A_k + e_{ijkl}$, where μ = the overall mean, T_i = the fixed

effect of treatment, P_j = the fixed effect of period, A_k = the random effect of animal and e_{ijkl} = residual error. Significance was declared at $P < 0.05$.

4.3 Results

4.3.1 Chemical composition of the experimental diets

The DM and OM contents of the diets ranged from 88.8 to 91.3% and from 92.1 to 95.8%, respectively, and no significant differences were found in DM or OM among these four experimental diets (Table 4.2). The CP content of TKPER-R was significantly higher than that of SBCR-R and lower than that of SBM-R ($P < 0.05$). There was no significant difference observed in EE content between TKPER-R and the other three experimental diets, respectively. The aNDFom content of TKPER-R at 32.4% was lower than that of SB-R and SBCR-R ($P < 0.05$). The NFC content of TKPER-R, 28.4%, was significantly higher than that of SB-R ($P < 0.05$).

Table 4.2 Ingredients and chemical composition of the experimental diets (%)

Item	TKPER-R	SB-R	SBM-R	SBCR-R
Ingredients†				
SB	-	50.0	-	-
SBCR	-	-	-	50.0
SBM	-	-	50.0	-
TKPER	50.0	-	-	-
Ryegrass straw	50.0	50.0	50.0	50.0
Chemical composition				
Dry matter	88.8	90.6	89.4	91.3
Organic matter†	92.1	94.7	93.7	95.8
Crude protein†	24.6^{b}	23.4^{b}	35.4^{c}	17.9^{a}
Ether extract†	6.7^{ab}	10.3^{b}	1.4^{a}	5.5^{ab}
aNDFom †	32.4^{a}	40.3^{b}	34.3^{a}	50.1^{c}
Crude ash†	7.9^{b}	5.3^{a}	6.3^{ab}	4.2^{a}
NFC‡	28.4^{b}	20.7^{a}	22.7^{ab}	22.3^{ab}

Means in the same row with different superscripts differ significantly ($P < 0.05$). † On a dry matter basis. ‡calculated by 100 - (CP + EE + aNDFom + CA). aNDFom, neutral detergent fiber exclusive of residual ash; NFC, nonfibrous carbohydrate. TKPER, tamarind kernel powder extract residue; SB, dry heat soybean; SBM, soybean meal; SBCR, dry soybean curd residue.

4.3.2 Digestibility

DM intake and digestibility of DM and OM were not different among treatments (Table 4.3). The CP digestibility of TKPER-R was significantly higher than SBCR-R ($P < 0.05$), but not from SB-R or SBM-R. No significant difference was observed among TKPER-R, SB-R and SBCR-R in EE digestibility and the EE digestibility of SBM-R was half of those of SB-R, SBCR-R and TKPER-R. The aNDFom digestibility of TKPER-R was lower than that of SBCR-R ($P < 0.05$). Meanwhile, NFC digestibility of TKPER-R was similar to that of SBCR-R and SBM-R, and higher than that of SB-R

($P < 0.05$).

Table 4.3 DM intake and apparent digestibility of the experimental diets in wethers

Item	TKPER-R	SB-R	SBM-R	SBCR-R	SEM
DM intake (g/day/$BW^{0.75}$)	47.6	48.6	47.9	49.0	0.30
Digestibility (%)					
Dry matter	57.0	56.5	60.2	59.9	0.42
Organic matter†	72.4	69.7	72.2	76.9	0.47
Crude protein†	87.0^{bc}	83.4^{ab}	90.7^{c}	80.3^{a}	0.59
Ether extract†	86.0^{b}	88.6^{b}	43.5^{a}	83.1^{b}	1.49
aNDFom †	49.0^{a}	54.8^{ab}	56.9^{ab}	64.2^{b}	3.07
NFC†	83.2^{b}	73.9^{a}	85.6^{b}	83.4^{b}	1.32

Means in the same row with different superscripts differ significantly ($P < 0.05$). SEM, standard error of means. † On a dry matter basis. aNDFom, neutral detergent fiber exclusive of residual ash; NFC, nonfibrous carbohydrate. TKPER-R, SB-R, SBM-R and SBCR-R, fed tamarind kernel powder extract residue, dry heat soybean, soybean meal and dry soybean curd residue with ryegrass straw, respectively, at 1:1 on a dry matter basis.

Table 4.4 shows the estimated digestibility of TKPER, SB, SBM and SBCR. The estimated digestibility and TDN of TKPER were calculated by subtracting the digestibility and TDN of ryegrass straw. The digestibility of ryegrass straw of DM, OM, CP, EE and NFC were 55.2%, 54.4%, 48.3%, 64.2% and 79.9%, respectively. The DM and OM digestibility of the test feeds ranged between 56.8 and 65.1%, and 84.7 and 98.8%, respectively. The differences observed among the feeds were not significant. The CP digestibility of the test feeds was quite high, above 90% for all, and the CP digestibility of TKPER was not significantly different from those of the other test feeds. The digestibility of EE and NFC in TKPER were 67.5% and 84.7%, respectively. The EE digestibility of TKPER was higher than that of SBM and lower than that of SB and

SBCR ($P < 0.05$), and NFC digestibility of TKPER was higher than that of SBCR ($P < 0.05$). The TDN content of test feeds ranged from 83.0 to 105.1%. The TDN content of TKPER was higher than that of SBM ($P < 0.05$).

Table 4.4 Estimated digestibility and total digestible nutrients of the test feeds (%)

Item	TKPER	SB	SBM	SBCR	SEM
Digestibility† (%)					
Dry matter	58.7	56.8	64.9	65.1	3.49
Organic matter‡	88.7	84.7	89.1	98.8	2.76
Crude protein‡	94.6^{ab}	91.0^{a}	99.9^{b}	94.5^{ab}	1.83
Ether extract‡	67.5^{b}	82.5^{c}	-3.3^{a}	83.2^{c}	2.69
NFC‡	84.7^{b}	71.8^{ab}	70.2^{ab}	59.9^{a}	2.35
TDN§	93.8^{b}	101.1^{b}	83.0^{a}	105.1^{b}	3.72

Means in the same row with different superscripts differ significantly ($P < 0.05$). SEM, standard error of means. †Estimated by subtracting the digestibility of ryegrass straw. ‡On a dry matter basis. §Estimated according to NARO 2010. TDN content = digestible OM content + digestible EE content*1.25. TKPER, tamarind kernel powder extract residue; SB, dry heat soybean; SBM, soybean meal; SBCR, dry soybean curd residue.

4.3.3 Nitrogen balance

As for nitrogen balance, the intake N, fecal N and urinary N levels of TKPER-R did not significantly differ from those of SB-R, SBM-R and SBCR-R. The retention N of TKPER-R was significantly lower than that of SBM-R ($P < 0.05$). There was no significant difference among treatments in N retention (% N intake).

Table 4.5 Nitrogen balance of wethers fed with the experimental diets

Item	TKPER-R	SB-R	SBM-R	SBCR-R	SEM
N balance(g/day/$BW^{0.75}$)					
N intake	1.88^{ab}	1.81^{a}	2.71^{b}	1.40^{a}	0.01
Fecal N	0.24	0.30	0.25	0.28	0.05
Urinary N	1.32^{ab}	1.20^{ab}	1.75^{b}	0.82^{a}	0.13
Retention N	0.31^{a}	0.32^{a}	0.71^{b}	0.30^{a}	0.05
N retention(% N intake)	16.79	18.02	26.13	21.46	3.36

Means in the same row with different superscripts differ significantly ($P < 0.05$). SEM, standard error of means. TKPER-R, SB-R, SBM-R and SBCR-R, fed tamarind kernel powder extract residue, dry heat soybean, soybean meal and dry soybean curd residue with ryegrass straw, respectively, at 1:1 on a dry matter basis.

4.3.4 Ruminal fermentation

No significant differences were found in the pH of ruminal fluid before feeding among the four treatments (Table 4.6). At 4 h after feeding, the pH of SBCR-R was lower than those of SB-R and TKPER-R ($P < 0.05$), but no significant differences were observed between TKPER-R, SB-R and SBM-R. As for the ruminal NH_3-N, SBCR-R was lowest and SBM-R was highest in concentration ($P < 0.05$) at both sampling times. The NH_3-N content in TKPER-R did not significantly differ from SB-R or SBM-R before feeding, or from SB-R and SBCR-R at 4 h after feeding.

The total VFA concentration (mmol/L) of each treatment before feeding was 66.9, 60.6, 78.8 and 77.6 for TKPER-R, SB-R, SBM-R and SBCR-R, respectively. No significant differences were found in the composition of acetate (C2), propionate (C3) and butyrate (nC4) contents, or in the C2:C3 ratio among treatments before feeding. At

4 h after feeding, there were no significant differences in total VFA concentration and C2 content among the treatments. The TKPER-R had higher C3 but lower nC4 contents and lower C2:C3 ratio than SBM-R ($P < 0.05$), whereas no significant differences in C3 content, nC4 content, or C2:C3 ratio were found between TKPER-R, SB-R and SBCR-R.

Table 4.6 Ruminal fermentation in wethers fed with the experimental diets at 0 and 4 h after feeding

Item	TKPER-R	SB-R	SBM-R	SBCR-R	SEM
Ruminal pH					
0 h	7.05	6.98	6.73	6.82	0.08
4 h	6.62^{b}	6.42^{b}	6.25^{ab}	5.92^{a}	0.10
NH_3-N (mgN/dL)					
0 h	47.95^{bc}	43.40^{ab}	61.25^{c}	31.50^{a}	3.71
4 h	43.58^{ab}	51.10^{b}	73.33^{c}	28.35^{a}	4.93
Total VFA(mmol/L)					
0 h	66.9	60.6	78.8	77.6	10.17
4 h	84.6	74.4	76.6	83.4	5.94
Acetate (C2) (%)					
0 h	52.9	53.6	54.5	57.2	1.12
4 h	50.2	51.1	52.1	54.0	1.29
Propionate (C3) (%)					
0 h	28.5	28.1	24.1	25.5	1.34
4 h	34.6^{b}	30.6^{ab}	24.8^{a}	32.4^{b}	1.32
Butyrate (nC4) (%)					
0 h	12.6	13.1	15.9	13.4	1.11
4 h	12.2^{a}	14.7^{ab}	18.8^{b}	11.8^{a}	0.98
C2:C3					
0 h	1.9	1.9	2.3	2.3	0.13
4 h	1.5^{a}	1.7^{ab}	2.1^{b}	1.7^{ab}	0.11

Means in the same row with different superscripts differ significantly ($P < 0.05$).

SEM, standard error of means.

TKPER-R, SB-R, SBM-R and SBCR-R, fed tamarind kernel powder extract residue, dry heat soybean, soybean meal and dry soybean curd residue with ryegrass straw, respectively, at 1:1 on a dry matter basis.

4.3.5 Blood metabolites

No significant difference was observed in Glu, TP, Alb, NEFA, AST, ALT, γ-GTP, Ca and IP concentrations among four treatments (Table 4.7). The T-Cho and PL concentrations of TKPER-R did not differ significantly compared to the other three treatments, respectively. The BUN concentration of TKPER-R was significantly higher than that of SBCR-R.

Table 4.7 Blood metabolites in wethers fed with the experimental diets

Item	TKPER-R	SB-R	SBM-R	SBCR-R	SEM
Glu（mg/dL）	60.50	59.50	65.25	62.75	2.69
TP（g/dL）	6.93	7.05	6.93	6.98	0.11
Alb（g/dL）	4.03	4.05	4.00	4.03	0.11
BUN（mg/dL）	36.35^{b}	34.43^{ab}	39.20^{b}	25.10^{a}	2.16
NEFA（mEq/L）	0.23	0.24	0.10	0.18	0.03
T-Cho（mg/dL）	100.25^{ab}	122.25^{b}	71.25^{a}	115.25^{b}	9.93
PL（mg/dL）	148.25^{ab}	176.00^{b}	95.75^{a}	168.75^{b}	12.72
AST（IU/L)	104.75	97.25	61.00	102.75	14.06
ALT(IU/L）	13.50	13.75	10.75	13.00	2.07
γ-GTP（IU/L）	68.25	58.75	65.50	62.50	5.41
Ca（mg/dL）	9.10	9.43	9.50	10.10	0.60
IP（mg/dL）	7.63	6.95	6.05	5.88	1.01

Means in the same row with different superscripts differ significantly ($P < 0.05$).

SEM, standard error of means.

Glu, glucose; TP, total protein; Alb, albumin; BUN, blood urea nitrogen; NEFA, nonesterified fatty acid; T-Cho, total cholesterol; PL, phospholipids; AST, aspartate aminotransferase; ALT, alanine transaminase; γ-GTP, γ-glutamyl transpeptidase; Ca, calcium; IP, inorganic phosphorus.

TKPER-R, SB-R, SBM-R and SBCR-R, fed tamarind kernel powder extract residue, dry heat soybean, soybean meal and dry soybean curd residue with ryegrass straw, respectively, at 1:1 on a dry matter basis.

4.4 Discussion

4.4.1 Chemical composition, digestibility and nitrogen balance

The CP contents of TKPER are similar to those of soybean, and slightly less than those of SBM; the EE contents are similar to those of SB and SBCR according to the NARO (2010). TKPER had high NFC content and no aNDFom content, which was one of its most important characteristics compared to the other test feeds. The high NFC content in TKPER was attributed to polysaccharide escaping from the extraction process for food additives, and the quite low aNDFom content was due to the alkaline treatment before extraction. In the diet of dairy cows, NFC is an important nutrient, as it can increase milk protein content and prevent laminitis (Looper, 2012). Increasing NFC diet content to 34.0-40.0% can increase rumen bacteria production, and consequently, milk protein yield (Looper, 2012).

The TKPER-R diet was ingested completely by wethers fed at 2% of BW on a DM basis and the DM intake was similar among the four treatments (Table 4.3), which indicated that TKPER was not less palatable for wethers than SB, SBM or SBCR. The CP digestibility of TKPER-R was higher than that of SBCR-R ($P < 0.05$), and the EE digestibility of TKPER-R was higher than that of SBM-R ($P < 0.05$), which might have resulted from its relatively higher CP and EE contents. The aNDFom digestibility of TKPER-R was significantly lower than that of SBCR-R. This was attributed to the non-detected aNDFom in TKPER (Table 4.1). The NFC digestibility of TKPER-R was higher than that of SB-R ($P < 0.05$), which could be explained by the higher NFC

content of TKPER-R ($P < 0.05$).

Regarding nitrogen balance, results implied that the TKPER-R diet, in which TKPER was mixed with ryegrass straw at a ratio of 1:1, did not have any effects on nitrogen balance, compared to the other experimental diets. The SBM feeding in SBM-R increased CP digestibility, excretion N in urine, retention N, and ruminal NH_3-N content in the present experiment, which was consistent with previous studies (Ahrar *et al.* 1979; Stern *et al.* 1994). The high CP content in SBM-R increased CP digestibility and ruminal NH_3-N content at 4 h after feeding. The highest urinary N in SBM-R suggested that excess nitrogen from the diet was mainly excreted in urine. The retention N, however, was not aggravated but improved by SBM feeding.

4.4.2 Ruminal fermentation

In general, ruminal pH decreases following ingestion due to ruminal fermentation. The value, however, is controlled between 5.5 and 7.0 (Bambi et al., 2011). The values of TKPER-R, 7.05 and 6.62, were not significantly different from the values of SB-R or SBM-R at both sampling times (Table 4.6). This suggested that the TKPER feeding did not have a negative effect on ruminal pH in wethers. Meanwhile, the ruminal pH in SBCR-R showed acute decrease and was lower than that in SB-R and TKPER-R ($P < 0.05$) at 4 h after feeding. The higher digestibility of aNDFom and NFC in SBCR-R compared to TKPER-R (aNDFom) and SB-R (NFC), respectively, might have produced this difference.

Higher ruminal C3 content and lower C2:C3 ratio at 4 h after feeding in wethers fed the TKPER-R diet (Table 4.6) were observed in this study. This was likely due to the relatively higher NFC content, digestibility, and lack of aNDFom content in TKPER. Bramley *et al.* (2008) indicated that high NFC percentage in diet provides conditions that favor the growth of bacteria fermenting sugars and starch. Combining these results with the lower ruminal NH_3-N content in wethers fed TKPER-R diet at 4 h after feeding compared to before feeding, it is likely that ruminal microorganisms could utilize protein and energy efficiently for their growth. Higher NFC diet could provide more fermentable energy in the rumen, which should reduce ruminal ammonia concentrations (Canna *et al.* 1998). In the present experiment, microorganisms could efficiently break down protein interacting with energy from TKPER and take advantage of NH_3-N faster after ingesting the TKPER diet.

4.4.3 Blood metabolites

The wethers exhibited no visible symptoms of metabolic disorders during the experiment. The concentration of Glu, TP, Alb, NEFA, Ca and IP were within the normal range of values (Glu, 44-81 mg/dL; TP, 6.0-7.9 g/dL; Alb, 2.7-3.7 g/dL; NEFA, < 0.30 mEq/L; Ca, 9.0-11.5 mg/dL; IP, 4.0-7.3 mg/dL; Kaneko *et al.* 2008). Solomon *et al.* (1992) reported that feeding diets with palm oil increased serum cholesterol. The higher EE contents in the experimental diets might have contributed to higher plasma T-Cho and PL concentration.

The wethers in each treatment had higher serum BUN concentrations than the normal range of values (10-26 mg/dL, Kaneko *et al.* 2008) since the CP contents in the experimental diets were high. SBCR-R had the lowest BUN due to its lower CP content, because BUN is affected by dietary levels of protein (Preston *et al.* 1965). The concentrations of AST, ALT and γ-GTP in plasma are indicators of liver functions. The concentrations among the four treatments did not show significant differences and were within the range of normal values (AST, 307 IU/L; ALT, 30 IU/L; γ-GPT, 17-69 IU/L, Kaneko *et al.* 2008). Hence, we concluded that liver function in the experimental wethers was normal.

4.5 Conclusion

The present results indicate that TKPER did not have any side effect on digestibility, nitrogen balance and ruminal fermentation in wethers. It could be concluded that TKPER can be used as a high-protein source feed to be substituted for an expensive ingredient for growing livestock, or as a high-NFC nutrition energy source feed for lactating and fattening livestock. Due to the absence of fiber in TKPER, appropriate roughage source should be chosen together with TKPER to meet the fiber requirement of ruminants.

CHAPTER 5

General conclusion

5.1 The nutrition characteristics of TKPER

The TKPER is a by-product of the processing of polysaccharide thickener. Numerically 400 tons of TKPER are produced annually in one of the major factories in Japan. It will be helpful to decrease feed cost and improve self-sufficiency in Japan if it could be used as a by-product feed in animal production.

In chapter 2 the *in vitro* gas production, DM and CP digestibility of TKPER were investigated comparing to the traditional soybean product and by-products, i.e., SB, SBM and SBCR. The results indicated that TKPERs had high contents of CP, EE and NFC, although it was not rich in aNDFom or ADFom. TKPERs produced lower gas production in each observation time and had similar VFA contents to SBM and SBCR, and higher NH_3-N contents to SBCR.

In chapter 3 the nylon bag technique was used to evaluate *in situ* DM and CP degradation in wethers. Since both tamarind and soybean belongs to leguminous family, and production processes of TKPER and SBM are similar, the CP content of TKPER was similar to that of SBM, which may have led to the similar performance in *in situ* CP degradability. It could be indicated that TKPER could be used as a protein source feed as a substitute for the expensive ingredient: SBM.

In order to further understand the digestibility and ruminal characteristics of TKPER comparing to traditional soybean product and by-products, the digestibility, nitrogen balance and ruminal fermentation of TKPER were examined in *in vivo* experiment in chapter 4. The results demonstrated that TKPER was palatable for

wethers. Estimated DM and CP digestibility of TKPER did not differ compared to the other feeds. The estimated NFC digestibility and TDN contents of TKPER were 84.7% and 93.8%, respectively. Judging from digestibility, nitrogen balance and ruminal fermentation, TKPER did not show any side effect to wethers during the whole experiment period.

5.2 Economic evaluation of use of TKPER in Japanese beef fattening production system

Yamada *et al.* (unpublished data) evaluated the economic and environmental outputs of Japanese black steer production in order to examine optimum slaughter age by simulation using a bio-economic model. In this model, the input information included average daily gain (0.76 kg/d), mature weight (800 kg), initial and final weight of fattening (289 and 755 kg), mature BMS number (6), carcass unit price (1,591 yen/kg), composition of diets and price of each feed (yen/kg). Concentrates in diet were corn (39.5%), barley (32.0%), wheat bran (23.0%), SBM (5.0%), calcium carbonate (0.5%) and roughages in diet were hay (73.8%) and rice straw (26.2%) quoting a previous study (Ogino *et al.* 2004). To evaluate impact of replacement of an expensive ingredient in the concentrate, TKPER made place for SBM in the model. According to the model, the maximum profits day was around day 700 for steers fed with SBM or TKPER. However, the profits from steers fed with TKPER were much more than those from steers fed with SBM due to the much lower price of TKPER (25 *vs* 70.5 yen/kg).

The maximum profit per head was around 13,500 yen/year from the steer production using SBM while it would be 16,500 yen if SBM was replaced by TKPER. As to the environmental evaluation, the nitrogen surplus or greenhouse gas emission per head was similar from steers fed with SBM and TKPER by using this model.

On the other hand, Oishi *et al.* (2011) used a modified least-cost feed formulation method to optimize economic and environmental criteria in beef cattle fattening system by using various by-products, including brewer's grain, soybean curd residue, soy sauce cake and so on. The purchased concentrates using in formulation were calcium carbonate, corn, barley, SBM and wheat bran. According to this simulation, the only by-product feed was assumed as TKPER instead of SBM. We found that SBM was recommended to be totally replaced by TKPER in an optimized diet for beef fattening.

GENERAL SUMMARY

Utilization of by-products as animal feeds is of crucial importance in Japan not only because of decreasing feed cost but also improving self-sufficiency. TKPER is a by-product derived from the tamarind kernel powder that is obtained during the extraction of polysaccharides, which is widely used in the food industry in Japan. This research conducted *in vitro*, *in situ* and *in vivo* experiments respectively in wethers for evaluating nutrition values of TKPER as a new feed for ruminants.

Firstly, two types of TKPER (TKPER I and II), of which the CP, EE, aNDFom and NFC contents (%) were 41.4 and 42.0, 11.9 and 15.0, 1.4 and 0.5, 36.1 and 33.7, respectively, were used in *in vitro* experiment to examine the gas production and fermentation characteristics by comparing to the traditional soybean product and by-products, i.e., SB, SBM and SBCR. The results showed TKPERs had significantly lower gas production than the other test feeds at each observation time as a result of lower fiber contents. The *in vitro* DM and CP digestibility (%) of TKPER I and II were 67.7 and 64.9, and 64.5 and 58.0, respectively, significantly lower than those of SB and SBM.

In order to investigate DM and CP degradability of TKPER in rumen, we conducted the *in situ* experiment by using nylon bag technique. Four wethers (55.6 ± 4.6 kg) with ruminal cannula were fed concentrate and ryegrass straw at CP 12% and TDN 67.0% at 2% of BW on a DM basis. The results demonstrated that *in situ* DM degradation parameters, rapidly degradable fraction (*a*), slowly degradable fraction (*b*) and the rate constant for disappearance of *b* fraction (*c*), respectively, of TKPER were similar to those of SB. The *in situ* CP degradation parameters (*a*, *b* and *c*) of TKPER

were similar to those of SBM. *In situ* effective DM and CP degradability of TKPER were significantly higher than those of SBCR.

Furthermore, the digestibility, nitrogen balance and ruminal fermentation of TKPER were examined in *in vivo* experiment by comparing to SB, SBM and SBCR. Four wethers (51.6 ± 5.5 kg) were assigned in a 4×4 Latin square design feeding TKPER, SB, SBM and SBCR with ryegrass straw (R) at a ratio of 1:1 at 2% of body weight in DM on a daily basis. The digestibility (%) of DM, CP and EE of TKPER-R diet were 57.0, 87.0 and 86.0, respectively. Higher NFC digestibility was observed in TKPER-R diet (83.2%) than in SB-R diet (73.9%). Wethers fed the TKPER-R diet had lower retention of nitrogen and ruminal ammonia nitrogen contents at 4 h after feeding than those fed the SBM-R diet, which had values similar to the SB-R or SBCR-R diet. The TKPER feeding had higher propionate and lower butyrate content, as well as lower acetate to propionate ratio in rumen fluid than SBM feeding at 4 h after feeding.

From this research it could be concluded that TKPER can be used as a high-protein source feed to be substituted for an expensive ingredient for growing livestock, or as a high-NFC nutrition energy source feed for lactating and fattening livestock. It is noted that due to the absence of fiber in TKPER, appropriate roughage source should be chosen together with TKPER to meet the fiber requirement of ruminants. Further study will be necessary to conduct the characteristic of TKPER used in an optimized diet for beef cattle or dairy cow.

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