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Degradation of tryptophan and related indolic compounds by ruminal bacteria, protozoa and their mixture in vitro

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Summary. In vitro experiments were conducted to examine the degradation of D- and L-isomers of tryptophan (Trp) and 10 related indolic compounds by mixed rumen bacteria (B), protozoa (P) and a combination of the two (BP). The analyses were carried out by HPLC. D-Trp (1.0 mM) was not degraded by rumen microorganisms during the 24-h incubation period. The net degradation of 1 mM L-Trp was 46.5%, 8.7% and 80.0% by B, P and BP suspensions, respectively. Trp was degraded into indoleacetic acid, indolelactic acid and indole by rumen bacteria and protozoa, and into skatole, *p*-cresol and indolepropionic acid by rumen bacteria only. Of them, indoleacetic acid was the major product of Trp found in B (15.4%) and P (3.1%), and skatole in BP (43.2%). This is the first report of the production of indolelactic acid and *p*-cresol from Trp by rumen microbes. Starch, D-glucose, salinomycin and monensin inhibited the production of skatole and indole from Trp, and skatole from indoleacetic acid by rumen bacteria.

Keywords: Tryptophan degradation – Skatole – P-cresol – Indoleacetic acid – Rumen bacteria – Rumen protozoa

Introduction

Previous studies on the ruminal degradation of tryptophan have revealed that several metabolites are formed by ruminal microorganisms. Indole (IND), indoleacetic acid (IAA) and indolepropionic acid (IPR) were detected in washed cell suspensions of ruminal microorganisms, when incubated with tryptophan (Trp) (Lacoste, 1961). Short incubations of L-[3-methylene-^{14}C] Trp with ruminal microorganisms resulted in the incorporation of the radioactivity into IAA (Scott et al., 1963). IND and skatole (SKT) were also formed in vitro and in vivo from DL-Trp (Lewis and Emery, 1962b, c). Tryptamine (TPM) has also been detected in in vitro incubations of DL-Trp with ruminal microorganisms (Schatzman and Gerber, 1972). Several studies also reported the natural occurrence of low concentrations of IND and SKT in ruminal fluid (Cappa, 1955; Lewis and Emery, 1962c; Spisna and Cappa, 1954). Incubation of L-[U-benzene ring-^{14}C] Trp with ruminal microorganisms for 24 h resulted in 39% of the added radioactivity being incorporated into SKT, 7% into IND and 4% into IAA (Yokoyama and Carlson, 1973).

In order to examine Trp metabolism by rumen microorganisms in more detail, we developed a HPLC method that could separate eleven compounds, including Trp, indolelactic acid (ILA), tryptophol (TPP), TPM, indole-3-acetaldehyde (ICA), IAA, indole-3-aldehyde (ILD), SKT, IND, *p*-hydroxyphenylacetic acid (HPA) and *trans*-cinnamic acid (CNM) simultaneously (Mohammed et al., 1998a). In the course of the experiment, however, it was found that the HPLC method (Mohammed et al., 1998a) could not separate Trp from an unidentified compound (X1) naturally produced from indolepyruvic acid (IPA) (Mohammed et al., 1998b) after dissolution in 25% ethanol and even after incubation with MB9 buffer solution (Onodera and Henderson, 1980) which was used for protozoal incubation.

In the present study, in vitro experiments were conducted to investigate the degradation of D- and L-isomers of Trp by B, P and BP suspensions, analyzing both supernatants and hydrolyzates by HPLC (Mohammed et al., 1998a; Mohammed et al., 1998b). The degradation of 10 related indolic compounds by B, P and BP was also examined and their related products in the supernatants were also measured by HPLC (Mohammed et al., 1998a).

Materials and methods

Animals and their management

Three mature rumen fistulated goats (Japanese native breed) with a mean live weight of 35 ± 5 kg, fed lucerne (*Medicago sativa*) cubes (23 g DM/kg $BW^{0.75}$) and a concentrate mixture (8 kg DM/kg $BW^{0.75}$) in two equal portions, given at 9 am and 5 pm, were used. The goats were housed in individual pens under approximately constant environmental conditions. They had free access to fresh water.

Preparation of rumen microbial suspensions

Rumen contents were obtained from the fistulated goats before morning feeding and were strained through four layers of surgical gauze into a separating funnel which was gassed with a mixture of 95% N_2 and 5% CO_2, passed through a heated (250°C) copper furnace to remove O_2. The contents were incubated at 39°C for about 60 min to allow feed debris to float. The suspensions of mixed bacteria (B), mixed protozoa (P) and B plus P (BP) were prepared as previously described (Mohammed et al., 1999a). The P suspensions always included 0.1 mg/ml each of chloramphenicol, streptomycin sulfate and penicillin G potassium to suppress the biochemical activities of the contaminating bacteria. Protozoal compositions ($\times 10^4$/ml) in BP and P were 94.5 and 163.1, 5.6 and 11.2, and 1.7 and 3.0 for *Entodiniinae*, *Diplodiniinae* and *Isotrichidae*, respectively.

Incubations were conducted in 30-ml Erlenmeyer flasks fitted with rubber sleeves. The substrates (20 mM each) were dissolved in 50% ethanol and 1 ml of each substrate was added to the incubation flask along with 19 ml of B, P and BP suspensions. Each flask was flushed with O_2-free 95% N_2 and 5% CO_2 and incubated at 39°C for 24 h. Samples (1 ml) were collected at 0, 12 and 24 h, mixed with an equal volume of 4% (w/v) sulfosalicylic acid for deproteinization, and centrifuged at 27,000 g for 20 min at 4°C. The supernatant fluid was filtered (0.45 μm pore size) and stored at 4°C. Pellets (mostly microorganisms) were hydrolyzed with 4 M LiOH at 110°C for 20 h (Lucus and Sotelo, 1980).

In order to determine protozoal numbers, 0.5-ml portions of the microbial suspensions were collected, mixed with 4.5 ml of methylgreen-formalin-salt (MFS) solution (Onodera et al., 1977) and kept at room temperature. Rumen protozoa were counted using a Fuchs-Rosenthal hemocytometer. Microbial nitrogen was determined using the Kjeldahl method (Helrich, 1990).

Analytical methods

The mobile phase used for isocratic elution was a mixture of methanol and 50 mM sodium acetate buffer (pH 4.2). The flow rate was 1.0 ml/min; column temperature, 40°C; monitoring wavelength at 280 nm with UV absorbance detector; column, LiChrospher 100 RP-18 (250 × 4 mm I. D.) of 5 μm particle size. The HPLC method of Mohammed et al. (1998a) with a methanol : acetate buffer ratio of 30 : 70 (v/v) was used for the analysis of Trp in the hydrolyzates, and its related compounds in the supernatants produced from D- and L- isomers of Trp by B, P and BP suspensions during 24-h incubation period. This method could also separate *p*-cresol (CRL), indoleacetamide (IAM) and indolepropionic acid (IPR) simultaneously with other compounds. Another HPLC method (Mohammed et al., 1998b) with a methanol : acetate buffer ratio of 20 : 80 (v/v) was used only for the analysis of remaining Trp in the supernatants after incubation with B, P and BP suspensions, because IPA, one of the metabolites of Trp breakdown (Yokoyama and Carlson, 1973) and the unknown peak (X1), produced from IPA did not separate from Trp when analyzed by the first HPLC method. Compounds were tested in triplicate, and fermentations were repeated by collecting rumen samples from each of three goats on three separate days. The values of all the compounds found in the supernatants and the hydrolyzates were expressed as the means of nine determinations and standard deviations of the differences between incubation with and without substrates.

Results

Net degradation of L-Trp and production of indolic compounds

"Degradation" was expressed here as either apparent or net degradation of Trp. Apparent degradation means the disappearance of Trp in the extracellular medium during incubation with rumen microorganisms. Trp (1 mM) was apparently degraded by 45.0 and 59.8%, 3.1 and 4.7%, and 53.3 and 86.3% in B, P and BP suspensions after 12 and 24-h incubations, respectively (Table 1). Average microbial nitrogen measurements (mg N/ml) in B, P and BP suspensions were 0.965 ± 0.052, 0.997 ± 0.079 and 1.768 ± 0.101, respectively. When the degradation rate was expressed with a unit of "per microbial nitrogen (MN)", the apparent Trp degradation rates were to be 38.8, 2.6 and 25.1 (from 0–12 h), and 25.8, 2.0 and 20.3 (from 12–24 h) μmol/g MN/h in B, P and BP, respectively. About 9.1 and 13.2%, and 4.8 and 6.3% of Trp was incorporated into cell protein by B and BP suspensions, and 25.1 and 39.8 μM Trp was liberated into the medium by P after 12 and 24 h incubation, respectively (Table 1). Therefore, the amount of Trp incorporated by B and BP, and liberated by P should be subtracted from those of the apparently disappeared Trp in the medium by B, P and BP suspensions. In fact, 35.9 and 46.5%, 5.6 and 8.7%, and 48.6 and 80.0% of Trp were degraded by B, P and BP after 12 and 24-h incubations, respectively (Table 1). The net degradation rates of Trp were 31.0, 4.7 and 22.9 (0–12 h), and 20.1, 3.6 and 18.8 (12–24 h) μmol/g MN/h in B, P and BP respectively.

IAA, ILA, IND, SKT, CRL and IPR were formed from Trp in the supernatants by B, P and BP (Table 1). As a whole, 14.3 and 15.4, 1.7 and 3.1, and 12.4 and 7.6% of Trp was converted to IAA by B, P and BP suspensions after 12 and 24-h incubations, respectively. The IAA production rate from Trp was 12.3, 1.4 and 5.8 (0–12 h), and 6.6, 1.3 and 1.8 (12–24 h) μmol/g of MN/h by B, P and BP suspensions, respectively. In total, 3.2 and 4.5, 1.0 and 2.4, and 4.1 and 5.4% of Trp was converted to ILA by B, P and BP suspensions

Table 1. Degradation of Trp and production of related compounds in the supernatants by mixed ruminal bacteria (B), protozoa (P) and the combination of B plus P (BP) after 12 and 24 h incubations

Compound		Mixed ruminal bacteria (B)		Protozoa (P)		Bacteria + Protozoa (BP)	
		12 h	24 h	12 h	24 h	12 h	24 h
		Concentration (μM)*					
Trp	Supernatant	550 ± 27	402 ± 21	969 ± 10	953 ± 7	467 ± 28	137 ± 10
	Pellet hydrolyzate	91 ± 11	132 ± 13	−25 ± 6	−40 ± 7	48 ± 6	63 ± 9
	Net degradation	359	465	56	87	486	800
Indoleacetic acid	Supernatant	143 ± 13	154 ± 16	17 ± 4	31 ± 4	124 ± 12	76 ± 13
Indolelactic acid	Supernatant	32 ± 9	45 ± 11	10 ± 6	24 ± 8	41 ± 9	54 ± 11
Indole	Supernatant	34 ± 7	46 ± 8	20 ± 5	27 ± 7	55 ± 9	89 ± 10
Skatole	Supernatant	35 ± 5	86 ± 7	0	0	132 ± 14	432 ± 20
p-Cresol	Supernatant	41 ± 7	48 ± 7	0	0	54 ± 9	75 ± 11
Indolepropionic acid	Supernatant	12 ± 5	18 ± 6	0	0	8 ± 3	14 ± 4

Initial concentration of Trp : 1 mM. * Mean values ± SD

respectively. The ILA production rate from Trp was 2.8, 0.8 and 1.9 (0–12 h), and 1.9, 1.0 and 1.3 (12–24 h) μmol/g of MN/h by B, P and BP suspensions, respectively. In the present experiment, we found that about 3.4 and 4.6, 2.0 and 2.7, and 5.5 and 8.9% of Trp was converted to IND by B, P and BP suspensions respectively. The IND production rate from Trp was 2.9, 1.7 and 2.6 (0–12 h), and 2.0, 1.1 and 2.1 (12–24 h) μmol/g of MN/h by B, P and BP suspensions respectively. SKT was also produced from Trp by rumen bacteria. About 3.5 and 8.6, 13.2 and 43.2% of Trp was converted to SKT by B and BP after 12 and 24-h incubations, respectively. P suspension had no ability to produce SKT from Trp. The SKT production rate from Trp was 3.0 and 6.2 (0–12 h), and 3.7 and 10.2 (12–24 h) μmol/g of MN/h by B and BP suspensions respectively. A considerable amount of CRL was produced from Trp by rumen bacteria. About 4.1 and 4.8, 5.4 and 7.5% of Trp was converted to CRL by B and BP suspensions after 12 and 24-h incubations, respectively. P suspension has no ability to produce CRL from Trp. The CRL production rate from Trp was 3.5 and 2.5 (0–12 h), and 2.1 and 1.8 (12–24 h) μmol/g of MN/h by B and BP suspensions, respectively. Small amounts of IPR were also produced by deamination of Trp. About 1.2 and 1.8, and 0.8 and 1.4% of Trp was converted to IPR by B and BP respectively. P did not produce IPR from Trp.

Degradation of indolic compounds

Degradation of IAA, ICA, ILD and IAM was demonstrated (Table 2). When IAA was used as a substrate, 13.5 and 20.2, and 34.6 and 53.2% of IAA was converted to SKT by B and BP after 12 and 24-h incubations, respectively. SKT was also produced from ICA, ILD and IAM via IAA by B and BP. ICA was completely converted to IAA by B, P and BP suspensions within 12 h. About 4.3% of SKT was produced in B from ICA after 24-h incubation only, whereas in BP, the amounts were 4.8 and 16.9% after 12 and 24-h incubations, respectively. P suspension did not produce SKT from ICA. On the other hand, 71.9 and 93.6, 12.5 and 15.2, and 54.7 and 83.6% of ILD were converted to IAA by B, P and BP suspensions after 12 and 24-h incubation periods, respectively. A small amount of SKT (2.1%) was also produced in B from ILD after 24-h incubation only, whereas in BP the amounts were 1.6 and 13.8% after 12 and 24-h incubations, respectively. P suspension did not produce SKT from ILD. About 60.4 and 65.3%, and 94.2 and 83.8% of IAM was also converted to IAA by B and BP after 12 and 24-h incubations, respectively. A small amount of SKT (2.8%) was found in B after 24-h incubation only, whereas in BP the amounts were 3.6 and 15.5% after 12 and 24-h incubations, respectively. ILA, TPP, TPM and IPR were not degraded by B, P and BP during the 24 h incubation period under the present experimental conditions. IND and SKT concentrations decreased slowly, when incubated with B and BP suspensions, though we did not find any related compound separated by this chromatogram as their by-products.

Table 2. Related compounds produced from indolic compounds in the supernatants by mixed ruminal bacteria (B), protozoa (P) and their mixture (BP) after 12 and 24 h incubations

Compound	Mixed ruminal bacteria (B)		Protozoa (P)		Bacteria + Protozoa (BP)	
	12 h	24 h	12 h	24 h	12 h	24 h
	Concentration (μM)*					
	⟨When indoleacetic acid (1 mM) was added as substrate⟩					
Indoleacetic acid	865 ± 23	798 ± 21	995 ± 14	993 ± 14	654 ± 22	468 ± 13
Skatole	135 ± 14	202 ± 14	0	0	346 ± 18	533 ± 21
	⟨When indoleacetaldehyde (1 mM) was added as substrate⟩					
Indoleacetaldehyde	0	0	0	0	0	0
Indoleacetic acid	987 ± 17	948 ± 12	988 ± 18	995 ± 12	942 ± 14	825 ± 22
Skatole	0	43 ± 6	0	0	48 ± 4	169 ± 13
	⟨When indolealdehyde (1 mM) was added as substrate⟩					
Indolealdehyde	273 ± 17	39 ± 4	870 ± 25	838 ± 19	429 ± 15	0
Indoleacetic acid	719 ± 21	936 ± 12	125 ± 10	153 ± 10	546 ± 18	836 ± 28
Skatole	0	21 ± 4	0	0	16 ± 3	138 ± 12
	⟨When indoleacetamide (1 mM) was added as substrate⟩					
Indoleacetamide	388 ± 20	329 ± 18	995 ± 9	991 ± 11	44 ± 26	0
Indoleacetic acid	605 ± 16	653 ± 21	0	0	942 ± 18	839 ± 26
Skatole	0	28 ± 4	0	0	36 ± 5	156 ± 14

* Mean values ± SD

Effects of starch, D-glucose, salinomycin and monensin on the formation of SKT and IND from Trp, and SKT from IAA

Two percent of starch and glucose, salinomycin (SL) and monensin (MN) tested at the 5 and 7.5 μg/ml level, respectively, inhibited the production of SKT from Trp and IAA by B and BP suspensions, and IND from Trp by B, P and BP suspensions (Table 3). In contrast, IAA production increased. In the present experiment, salinomycin was most effective for the inhibition of SKT and IND production from Trp, and SKT from IAA. About 85.6 and 82.6%, and 84.4 and 68.3% of SKT and IND produced from Trp were inhibited by SL in B and BP suspensions during the 24 h incubation period, respectively. On the other hand, MN inhibited SKT and IND production from Trp by 76.8 and 78.2, and 81.6 and 61.3% in B and BP suspensions, respectively. SL and MN also inhibited the SKT production from IAA by 80.9 and 68.6%, and 77.7 and 64.9% in B and BP suspensions after 12 and 24 h, respectively.

Discussion

Trp is one of the amino acids that is relatively slowly metabolized by ruminal microbes (Candlish et al., 1970; Lewis and Emery, 1962a; Sirotnak et al., 1953). D-Trp was not degraded by B, P and BP during 24-h incubation period under the present experimental conditions. This observation is in agreement with previous workers who reported a similar effect using rumen microorganisms (Yokoyama and Carlson, 1973). When Trp was incubated with B and BP suspensions, a portion of it was incorporated into amino acid pool of the microbial cell and the microbial cell protein, whereas P suspension liberated endogenous Trp into the medium. This observation is in agreement with our previous results concerning Trp biosynthesis from indolepyruvic acid (Mohammed et al., 1999a), and indole and L-serine metabolism by rumen bacteria and protozoa (Mohammed et al., 1999b). Net degradation of Trp in B was 6.6 and 5.6-fold more than in P after 12 and 24-h incubation periods, respectively. This is the first report in which the net degradation ability of Trp by rumen protozoa and bacteria has actually been demonstrated as far as we are aware. The lower degradation rate of Trp in BP compared to B may be due to the predation of bacteria by protozoa. It is known that protozoa always engulf bacteria in the rumen ecosystem. The continuous engulfment of bacteria by protozoa can lower the number of bacteria and decrease the total bacterial activities to degrade Trp to related compounds. In contrast, a higher deaminase activity in P than B was found in cell-free extracts (Hino and Russell, 1985). The ciliate protozoa, particularly small entodinia, were more active in deaminase activities of

Table 3. Effect of antibiotics, D-glucose and starch on the formation of tryptophan metabolites by mixed ruminal bacteria (B), protozoa (P) and their combination B plus P (BP) after 12 and 24 h incubations

	Mixed ruminal bacteria (B)		Protozoa (P)		Bacteria + Protozoa (BP)	
	12 h	24 h	12 h	24 h	12 h	24 h
	Skatole concentration (μM)*					
	⟨When Trp (1 mM) was added as substrate⟩					
Control	35 ± 5	86 ± 7	0	0	132 ± 14	432 ± 20
2% Starch	31 ± 5	58 ± 7	0	0	95 ± 10	224 ± 15
2% Glucose	29 ± 6	51 ± 7	0	0	79 ± 9	102 ± 7
Salinomycin (5 μg/ml)	0	12 ± 5	0	0	24 ± 6	67 ± 9
Monensin (5 μg/ml)	0	20 ± 4	0	0	38 ± 7	80 ± 10
	⟨When indoleacetic acid (1 mM) was added as substrate⟩					
Control	135 ± 14	202 ± 14	0	0	346 ± 18	533 ± 21
2% Starch	79 ± 9	65 ± 7	0	0	157 ± 11	327 ± 18
2% Glucose	52 ± 7	54 ± 6	0	0	144 ± 10	291 ± 17
Salinomycin (5 μg/ml)	31 ± 5	39 ± 5	0	0	91 ± 9	167 ± 12
Monensin (5 μg/ml)	38 ± 6	45 ± 6	0	0	112 ± 9	187 ± 12
	Indole concentration (μM)*					
	⟨When Trp (1 mM) was added as substrate⟩					
Control	34 ± 7	46 ± 8	20 ± 5	27 ± 7	55 ± 9	89 ± 10
2% Starch	5 ± 2	9 ± 3	3 ± 2	5 ± 2	28 ± 5	41 ± 9
2% Glucose	3 ± 1	6 ± 2	0	3 ± 1	25 ± 5	38 ± 8
Salinomycin (5 μg/ml)	0	8 ± 3	0	0	14 ± 5	28 ± 8
Monensin (7.5 μg/ml)	0	10 ± 3	0	0	19 ± 6	35 ± 7

* Mean values ± SD

protein and peptide than rumen bacteria (Wallace et al., 1987). In this regard, Forsberg et al. (1984) detected lower deaminase activity in the autolysate of protozoa than bacteria. In the present experiment, we used washed cell suspensions of protozoa and bacteria, and the net degradation (mainly deamination) was lower in P than B, similar to the results obtained by Amin et al. (1997a) during degradation of phenylalanine by ruminal bacteria, protozoa and their mixture in vitro.

We did not determine IPA produced from Trp, because it was an unstable compound and produced some unidentified peaks as mentioned above. Production of IAA from Trp by mixed rumen microorganisms (Lacoste, 1961; Scott et al., 1963; Scott et al., 1964; Yokoyama and Carlson, 1973) and intestinal bacteria (Yokoyama and Carlson, 1979) have been reported. In the present experiment, we found that 7.6% of Trp was converted to IAA in BP during the 24 h incubation, compared to the value (4%) reported by Yokoyama et al. (1973) during 24-h incubation of radioactive tryptophan (9.8×10^{-4} M) with rumen microorganisms in vitro. The ability of B to produce IAA from Trp was 8.8 and 5.1-fold more than from P. The reason for the lower production rate of IAA from Trp in BP than B was same as mentioned before. Production of ILA from indolepyruvic acid in B and P (Mohammed et al., 1999a), and phenyllactic acid from phenylalanine and phenylpyruvic acid in B were reported previously (Amin and Onodera, 1997a, b). Formation of ILA from Trp was the first report in B and P. In the present study, the ability of B to produce ILA from Trp was 3.5 and 1.9-fold more than from P after 12 and 24-h incubation periods, respectively. A wide range of bacterial species such as *Escherichia coli*, *Proteus vulgaris*, *Paracolobactrum coliforme*, *Achromobacter liquefaciens* and *Micrococcus aerogenes* are capable of producing IND from Trp (Demoss and Moser, 1969). The production of IND from Trp has been found with mixed rumen microorganisms in vitro (Lewis and Emery, 1962a) and in vivo (Lewis and Emery, 1962c), in pure cultures of rumen bacteria (Elsden et al., 1976), and in ruminal fluid (Candlish et al., 1970; Lewis and Emery, 1962c; Yokoyama and Carlson, 1973). In the present experiment, we found that 8.9% of Trp was converted to IND in BP after

24-h incubation, which is close to the value (7%) reported by Yokoyama et al. (1973) during 24-h incubation of radioactive Trp (9.8×10^{-4} M) with rumen microorganisms in vitro. The ability of B to produce IND from Trp was 1.7 and 1.8-fold more than from P after 12 and 24-h incubation periods, respectively. Usually, the IND production rate in BP is lower than B, because protozoa always engulf bacteria. However, in the present experiment, the production rate was approximately equal. Therefore, a complex interaction exists between B and P. SKT was the major product of Trp in BP, which supports the result of previous worker (Carlson et al., 1972). Rumen protozoa can not produce SKT from Trp. This observation is in agreement with the previous report using P suspension from the rumen origin (Mohammed et al., 1999a; Mohammed et al., 1999b). The ability of BP to produce SKT from Trp was 2.1 and 2.8-fold more than from B after 12 and 24-h incubation periods, respectively, though P have no ability to produce SKT from Trp. In this connection, SKT production from Trp by mixed rumen microorganisms (Yokoyama and Carlson, 1973), rumen bacteria (*Lactobacillus* sp.) (Yokoyama et al., 1977) and intestinal bacteria (Yokoyama and Carlson, 1979) have been reported. In the present experiment, we also found that 43.2% of Trp was converted to SKT in BP after 24 h incubation, which was also close to the value (39%) reported by Yokoyama and Carlson (1973) during 24-h incubation of radioactive Trp (9.8×10^{-4} M) with rumen microorganisms in vitro. This is the first report to indicate that rumen bacteria can produce CRL from Trp. In this regard, the production of CRL from tyrosine via *p*-hydroxyphenylacetic acid (HPA) by skatole-producing *Lactobacillus* sp. (Yokoyama et al., 1977), and *Clostridium difficile* and *Clostridium scatologenes* (Elsden et al., 1976) have been reported. On the other hand, it has been reported that small amounts of Trp were produced from HPA by rumen microorganisms (Kristensen, 1974). Therefore, there may be a complex relationship between Tyr and Trp metabolism. The biological effects of IPR in ruminants are unknown, but it is a potent hydroxyl radical scavenger in rat brain (Poeggeler et al., 1999). Stack and Cotta (1986) reported that phenylpropionic acid, one of the metabolites of Phe, increased cellulose digestion by rumen microorganisms. A small amount of IPR was also produced from Trp which confirmed the results of Lacoste (1961). Elsden et al. (1976) also reported the formation of IPR from Trp by several clostridial species, including *Clostridium sporogenes*, *Clostridium botulinum* and *Clostridium caloritolerans*.

SKT was also produced from IAA. This is in agreement with the results of previous workers (Carlson et al., 1972; Yokoyama and Carlson, 1973). Yokoyama and Carlson (1973) found that 38% of the added radioactivity was incorporated into skatole during 24-h incubation of radioactive IAA (1.1×10^{-3} M) with rumen microorganisms in vitro, which is different from our results mentioned above. ICA and ILD was degraded to IAA and SKT, different from the results reported by Yokoyama and Carlson (1973) who qualitatively determined IAA and TPP from ICA, and IND and SKT from ILD by rumen microorganisms in vitro during the 24 h incubation period. Here, we report the first finding of the production of IAA from IAM. BP suspension was converted the IAM to IAA more rapidly than B, though P suspension had no ability to produce IAA from IAM. Therefore, a complex interaction must exist between B and P.

In the present study, ILA, TPP, TPM and IPR were not degraded by B, P and BP during the 24 h incubation period. Yokoyama and Carlson (1973) found similar results during degradation of TPP and TPM, but they found SKT and IND formed from ILA by rumen microorganisms in vitro, different from our results. This is the first report to show that IPR was not degraded by rumen bacteria and protozoa. Kohda et al. (1997) reported that anaerobic microorganisms isolated from porcine or chicken manure decomposed indole and skatole in composting processes. In the present study, IND and SKT concentrations decreased slowly when incubated with B and BP suspensions. They may be decomposed or produced different compounds from those compounds separated by our chromatogram. In a previous paper (Mohammed et al., 1999a), we reported that Trp was synthesized from IPA and other related compounds such as ILA, IAA, IND, SKT and CRL were produced by rumen microorganisms. Based on the indolic compounds that are degraded by B, P and BP to identifiable metabolites, it is possible to derive a tentative scheme for the fermentation pathways occurring in ruminal fluid in vitro (Fig. 1).

Yokoyama and Carlson (1973) reported that antibiotics such as chlorotetracycline, kanamycin, neomycin, oleandomycin, oxytetracycline, penicillin G, polymyxin B and streptomycin inhibited IND and SKT production from Trp and IAA by rumen microorganisms. In the present study, we also observed that SL

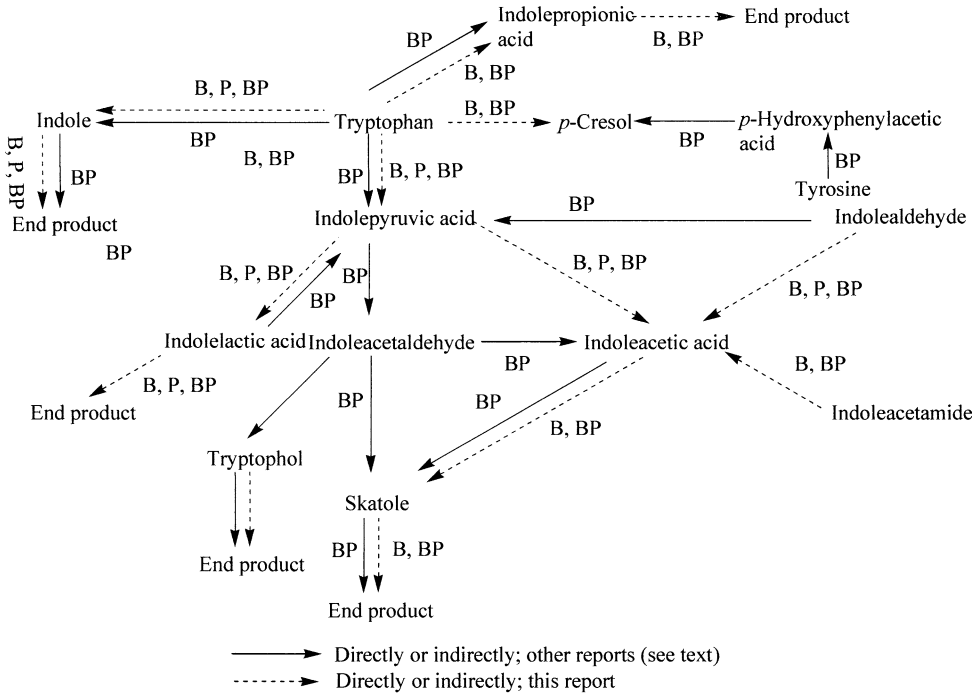

Fig. 1. Tentative pathway for the fermentation of L-tryptophan and related indolic compounds by mixed rumen bacteria (*B*), protozoa (*P*) and B plus P (*BP*)

and MN inhibited SKT production from Trp and IAA by B and BP, and IND production from Trp by B, P and BP suspensions. Glucose was potentially more active for the inhibition of SKT production from Trp and IAA by B and BP, and IND production from Trp by B, P and BP suspensions than that of starch by rumen microorganisms. Perhaps, at first, starch was degraded into glucose which in turn inhibited IND and SKT production by rumen microorganisms. Previous studies demonstrated that the formation of indole by tryptophanase can be induced by tryptophan and repressed by glucose in pure cultures (Boyd and Lichstein, 1955; Evans et al., 1942). It is, therefore, conceivable that, analogous to indole formation, the formation of skatole may be induced by the appropriate substrate and repressed by glucose. Although ionophores effectively controlled the SKT and IND production, no attempt was made to assess their effects on the total microbial population in ruminal fluid in the present study.

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