Some Bovine proteins behave as dietary fibres and reduce serum lipids in rats.

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ABSTRACT

We examined the physiological importance of bovine dietary proteins in rats fed diets prepared from bovine Achilles' tendons and arteries. Rats were fed for 4 weeks, with 20% casein diet (CON), in comparison with two diets containing 15% casein and 5% of either bovine Achilles' tendons (AC) or arteries (AR) protein preparations. The serum total cholesterol concentration and non-HDL cholesterol level in the AR fed group were significantly lower (P<0.05) than those in the CON fed group at the end of the 4 week feeding period. The hepatic mRNAs were measured, and the HMG-CoA reductase mRNA level was significantly lower (P<0.05) in AR fed group compared to CON fed group. Total hepatic cholesterol concentration, in rats fed AC was significantly (P<0.05) higher than in the CON fed group. The serum triglyceride (TG) concentration and fatty acid synthase (FAS) mRNA level in AC and AR fed groups were significantly lower (P<0.05) compared to the CON fed group through out the feeding period. Faecal neutral sterol excretion was significantly (P<0.05) higher in the AC and AR fed groups compared to the CON fed group. The results of this study demonstrate that the some bovine dietary proteins have similar functions as dietary fibres which lowered the serum lipid concentration by enhancing faecal neutral sterol excretion, or suppressing lipid synthesis in the liver. Moreover, favourable amino acid compositions in AR and AC may also have an effect on low plasma lipid concentration in bovine protein diets fed groups.

Introduction

At present there is an increasing interest in the capacity of food and food components to reduce the risk of non infectious diseases related to diet. Dietary fibres have gained a considerable interest due to its favourable health effects. According to recent studies it has been proven that dietary fibre intake is inversely correlated with several cardiovascular disease factors, which supports its protective role against cardio vascular diseases and recommendations for its increased consumption (Lairon *et al*.2005, Erkkila & Lichtenstein, 2006).

From past studies it has been shown that some plant fibres have hypocholesterolemic effect; which may be due to fibre-induced alterations of intestinal absorption, intestinal or pancreatic hormone secretion, lipoprotein metabolism, bile acid metabolism, or fermentation by-products and their effects on hepatic cholesterol synthesis (Kay 1982). When animal dietary fibres are concerned, relatively a few studies has been carried out to investigate the effect on lipid metabolism. Normally, people believe that those who at a risk of coronary heart disease should consume less red meat due to adverse health impacts.

Bovine Achilles' tendons and arteries are waste products of the beef industry, which could be used as a potential source of functional protein for possible food applications due to their favourable amino acid composition and undigestible behaviour. This hypothesis may be supported by previous findings showing that dietary proteins with favourable amino acid composition reduced the serum cholesterol level in rats (Morita *et al*.1997,Gudbrandsen *et al*.2005). Undigestible behaviour of animal protein may be due to the abound collagen concentration as AC and AR contain abound glycine and proline concentrations (Schubert *et al.* 1974). In this study, we investigated the effect of some bovine dietary proteins on serum lipids, liver lipids, faecal lipids, bacterial

population in the colon, and hepatic mRNAs in rats.

Experimental methods adopted

Animals and diets

Male Crj / Wistar rats (5 weeks old) were purchased from Charles River Japan Inc. (Yokohama, Japan). They were housed individually in cages with free access to food and water. The animal facility was maintained on a 12 h light/dark cycle at a temperature of 23 ± 1 ºC and relative humidity of 60 ± 5 %. The rats were randomly assigned in to three groups (n=5). There were no significant differences in body weights and serum total cholesterol concentrations among groups at the beginning of the experiment. The composition of each diet is shown in Table 1. The experimental rats were fed for 4 weeks, with 20% casein diet, in comparison with two diets containing 15% casein and 5%of either bovine Achilles' tendons or arteries protein preparations. The AC and AR proteins were prepared as follows; first, raw bovine Achilles' tendons and arteries were washed and boiled to be defatted for 2 hours. The boiled Achilles' tendons and arteries were freeze-dried and milled at 300 mesh. The compositions of AC and AR were as follows (mg/g dry weight): moisture, 2.1 and 4.1; protein: 73.9 and 81.0; Lipid: 23.1 and 8.4; carbohydrate: 0.5 and 5.9; ash: 0.4 and 0.6, respectively. Total moisture, protein, lipid and carbohydrate content were determined by the procedure of the Association of Official Analytical Chemists (1990). Dietary cholesterol level in the AC and AR was determined (g/1kg of diet), and it was 0.1 (0.01%) and 0.06 (0.006%), respectively. The control group consisted of rats fed 200 g/kg of casein. The amino acid compositions of the AC, AR and casein were shown in the Table 2. The amino acid compositions were determined as follows; first 4 mg of AC and AR samples were hydrolyzed in 2 ml of 6 N HCL at 110 ºC for 24 h, vaccum dried and then reconstituted with 1 ml of 0.2 N HCl, filtered with 0.45μm diameter filter

(W-13-5, Tosoh, Japan) and analysed with a Hitachi-8700 amino acid analyser. The rats were allowed free access to food and water for 4 weeks. Body weight and food consumption were recorded weekly and daily, respectively. This experimental design was approved by the Animal Experiment Committee of Obihiro University of Agriculture and Veterinary Medicine. All animal procedures conformed to standard principles described in *Guide for the care and Use of Laboratory Animals* (National Research Council, 1985).

Analytical procedures

Blood samples (1 mL) were collected between 08.00 and 10.00 hours from the jugular veins of fasting rats anaesthetized by sodium pentobarbital. The samples were taken in to tubes without an anticoagulant. After the samples were allowed to stand at room temperature for 2 h, the sera were separated by centrifugation at 1500 ***g*** for 20 min. All faecal excretions were collected during the last 3 d of the experimental period (4 weeks). Faecal dry weight did not differ among groups. The rats were killed by ether inhalation, and the livers and caecum quickly removed, washed with cold saline (9g NaCl/L), blotted dry on filter paper, and weighed before freezing for storage.

Chemical analysis.

Total cholesterol, HDL-cholesterol, and triglyceride (TG) concentrations in the serum were determined enzymatically using commercially available reagent kits (assay kits for the TDX system; Abbott laboratory Co., Irving, TX). The non-HDL cholesterol concentration was calculated as follows: non-HDL cholesterol= total cholesterol–HDL-cholesterol.

Total lipids were extracted from liver and faeces by a mixture of chloroform/methanol (2:1,vol/vol) (Folch *et al.*1957). The neutral steroids in each lipid sample obtained by saponification were acetylated (Matsubara *et al*.1990) and

analysed by gas–liquid chromatography (GLC) using a Shimadzu 14A chromatograph (Kyoto, Japan) with a DB17 capillary column (0.25mm×30m; J&W Scientific, Folsom, CA) with nitrogen as the carrier gas. Acidic sterols in faeces were measured by GLC following the method of Grundy *et al.* (1965). Sterol balance was calculated as sterol balance = (faecal cholesterol+faecal coprostanol+faecal bile acids)-dietary cholesterol intake. A part of the caecal content was taken out into desalting water in a vial without exposure to air, and suspended. The suspension of caecum was deproteinized with perchloric acid and to form sodium salts of the short chain fatty acids (SCFAs). Individual SCFA was measured by GLC with a glass column (2000 x 3 mm) packed with 80–100 mesh chromosorb W-AW DMCS with H_3PO_4 (100 mL/L) as the liquid phase after adding H_3PO_4 by the procedure of Hara *et al.* (1994). Faecal nitrogen content was determined by Kjeldahl's method. Apparent digestibility of protein was calculated as apparent protein digestibility = (protein intake-faecal protein)/protein intake×100.

RNA isolation, reverse transcription-PCR, and Southern blot Analysis

Total RNA was isolated from the liver by the acid guanidium-phenol-choloroform method using Isogen (Nippon Gene, Tokyo, Japan; Chomczynski & Sacchi,1987). mRNA encoding the LDL receptor (LDL-R), cholesterol 7α-hydroxylase (CYP7A1), fatty acid synthase (FAS), HMG-CoA reductase, scavenger receptor type B1(SR-B1), sterol regulatory elementary binding protein (SREBP-1c), and GAPDH (used as an invariant control) were analysed by semi-quantitative RT-PCR and subsequent Southern hybridization of the PCR products with each inner oligonucleotide probe. Total RNA samples were treated with DNase RQ1 (Promega, Madison, WI, USA) to remove genomic DNA and subjected to RT-PCR by using Moloney murine leukemia virus reverse transcriptase (Gibco-BRL, Gaithersburg, MD, USA) and EX-Taq polymerase

(Takara, Tokyo, Japan) with LDL receptor primers of oligonucleotides (upstream primer, 5'-ATTTTGGAGGATGAGAAGCAG-3'; downstream primer, 5'-CAGGGCGGGGAGTGTGAGAA-3'), CYP7A1 primers of oligonucleotides (upstream primer, 5'-GCCGTCCAAGAAATCAAGCAGT-3'; downstream primer, 5'-TGTGGGCAGCGAGAACAAAGT-3'), FAS primers of oligonucleotides (upstream primer, 5'-GCTGGAGCCCCTTTTTGTCTT-3'; down stream primer, 5'-ACCCCAGCACTGCAGTTTTCT-3'), HMG-CoA reductase primers of oligonucleotides (upstream primer, 5'-GCGTGCAAAGACAATCCTGGAG-3'; downstream primer, 5'-GTTAGACCTTGAGAACCCAATG-3'), SR-B1 primers of oligonucleotides (upstream primer, 5'-GTAGGGCCCAGAAGACACCAC-3'; downstream primer, 5'-CGCCTGCTTCACCACCTTCTT-3'), SREBP-1c primers of oligonucleotides (upstream primer, 5'-GAGCCACAATGAAGACCGCA-3'; downstream primer, 5'-CAAGGACAAGGGGCTACTCT-3'), and GAPDH primers of oligonucleotides (upstream primer, 5'-GCCATCAACGACCCCTTCATT-3'; downstream primer, 5'-CGCCTGCTTCACCACCTTCTT-3'). The reaction mixtures for the PCRs contained 25 pmol of each primer, 1.25 U of EX-Taq polymerase, 1 X PCR buffer (Takara), and 200 μM dNTP in a 50-μl reaction volume. Temperature cycling was as follows: first cycle, denaturation at 94°C for 3 min, annealing at 60°C for 1 min, and extension at 72°C for 2 min. Subsequent cycles were denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 2 min. The thermal cycling was completed by terminal extension at 72°C for 10 min. In total, 25 cycles were performed for LDL-R, CYP7A1, 30 cycles for HMG-CoA reductase, SR-B1, 20 cycles for FAS, GAPDH and SREBP-1c. Amplification products were electrophoresed on 2% agarose gel and transferred to a nylon membrane (Biodyne B, Pall Bio-Support, East Hills, NY, USA). Blots were hybridized with LDL-R probe of a

54-base oligoneucleotide (5’-CCAGGGTTGGTCGCTTTGCCCTGGAGCTGATCTGTCACCTCCAGCTCTCC CTC-3’), CYP7A1 probe of a 54-base oligonucleotide (5’-CCCGAAGGCCTGTTTAAGTGATGACTCTCAGCCGCCAAGTGACATCATCC AGTG-3’), FAS receptor probe of a 54-base oligonucleotide (5’-CTGCTCTCTGTGGATAGGACTGAATGCTGTGGCCTTCTGATAGACTCTTCT GGA-3’), HMG-CoA reductase probe of a 54-base oligonucleotide (5’-GATCTGTTGTGAACCATGTGACTTCTGACAAGATGTCCTGCTGCCAATGC TGCC-3’), SR-B1 probe of a 54-base oligoneucleotide (5’-TGCCGTGTGGACAGTGTGACATCTTGGGGCTCAGGACGTGGCACTGGCG GGTTG-3’), SREBP-1c probe of a 54-base oligonucleotide (5’-GCCGGCGTCTGAGGGTGGAGGGGTCAGCGTTTCTACCACTTCAGGTTTCA TGCC-3’), and GAPDH probe of a 54-base oligonucleotide (5’-TGATGACCAGCTTCCCATTCTCAGCCTTGACTGTGCCGTTGAACTTGCCG TGGG-3’). The probe was 3’-tailing labeled with digoxigenin, using a DIG oligonucleotide tailing kit (Boehringer Mannheim, Mannheim, Germany). Prehybridization, hybridization, and detection were carried out with a DIG luminescent detection kit (Boehringer Mannheim,Mannheim,Germany) as recommended by the manufacturer. The relative quantity of mRNA was estimated by densitometry scanning with x-ray film.

Growth of bacteria in the caecum

Coliform in the caecum was inoculated and grown for 2 days on DHL agar (Eiken Chemical Co., Ltd, Tokyo, Japan) plates at 37 °C. Anaerobe, *Lactobacillus* and *Bifidobacterium* in the caecum were incubated for 5 days on GAM agar (Nissui Pharmaceutical Co., Ltd, Tokyo, Japan), Rogosa agar (Merck KGaA, Darmstadt,

Germany) and BL agar (Eiken Chemical Co., Ltd, Tokyo, Japan) at 37 °C by the gaspak method according to the procedure of Mitsuoka *et al*. (1964,1965,1976).

Statistical Analysis

Data are presented as means and standard deviations for serum total cholesterol, HDL-cholesterol and non-HDL cholesterol at prescribed times. The significance of differences among treatment groups was determined by ANOVA with Duncan's multiple range test and student *t*-test (SAS Institute, Cary, NC, USA). Differences were considered significant at $P<0.05$.

Results

There was no significant difference in the food intake, body weight gain, liver weight, caecum weight, faecal dry weight and caecal pH, among groups at the end of the experimental period (data not shown). Apparent digestibility of AC and AR was around 92-93%, and which was lower than CON, 96%.

Table 3 shows the serum total cholesterol, non-HDL cholesterol, HDL-cholesterol and triglyceride concentrations of rats. The serum total cholesterol and non-HDL cholesterol concentration in the AR fed group were significantly lower than the CON group at the end of the 4 week feeding period. There was no significant difference in the HDL cholesterol concentration among the groups. The serum triglyceride concentration was significantly lower in AC and AR fed groups than that in the CON group through out the feeding period. The liver cholesterol concentrations in rats at the end of the experimental period are shown in Fig. 1. The liver cholesterol concentration in rats fed AC was significantly higher than in the CON group at the end of the 4 week feeding period.

Table 4 shows the dietary cholesterol intake, faecal neutral steroid and acidic steroid concentrations of rats. The bovine dietary cholesterol intake in rats fed AC and AR

diets were 7.20±1.03 and 6.91±0.61 μmol/rat/day, respectively. The faecal cholesterol and coprostanol concentrations were significantly higher in the AC and AR fed groups than those in the CON group, and those in the AC fed group were the highest among all the groups. However, the data on feacal coprostanone level was lacking in the Table 4 due to unavailability. Further, there were no significant differences in total bile acid, cholic acid, chenoceoxycholic acid, deoxycholic acid and lithocholic acid concentrations among the groups. Sterol balance in rats fed CON, AC and AR were 14.64, 26.96 and 14.16 μmol/rat/day, respectively. There were no significant differences in total SCFA, acetic acid, propionic acid and *n*-butyric acid excretions in caecum among 3 groups (data not shown).

The relative quantities of mRNA were determined by the Southern hybridization of PCR-amplified LDL-R cDNA, CYP7A1 cDNA, HMG-CoA reductase cDNA, SR-B1 cDNA, SREBP-1c cDNA, and FAS cDNA in the rat liver. The values of LDL-R, CYP7A1, HMG-CoA reductase, SR-B1, SREBP-1c and FAS mRNA were normalized to 100. There was no significant difference in relative quantity of LDL-R mRNA among 3 groups and the quantities for CON, AC, AR were 0.856, 809, 0.796 respectively. No significant difference was observed for the relative quantity of CYP7A1 mRNA among 3 groups (Fig.2). The relative quantity of hepatic HMG-CoA reductase mRNA in the AR fed group was significantly lower than the CON group ($P<0.05$), and that in AC fed group tended to be lower compared to the CON group(Fig.2). The relative quantity of FAS mRNA level in the AC and AR fed groups was significantly lower than that in the CON group (Fig.3). No significant difference was observed for the relative quantity of SR-B1 mRNA level, and the quantities for CON, AC and AR were 0.68, 0.67 and 0.66, respectively. The SREBP-1c mRNA level in AR fed group was significantly lower than that in the CON group, and that in the AC

fed group tended to be lower compared to the CON group (Fig.3). The FAS mRNA level was positively correlated with SREBP-1c mRNA level, the correlation coefficient (r) being 0.660 ($P < 0.01$) .

Table 5 shows the caecal bacterial population in rats. Anaerobe bacterial population was significantly higher in the AC and AR fed groups compared to the CON group. *Bifidobacterium* population in the AR fed group was significantly higher compared to the CON group, and that in the AC fed group tended to be elevated compared to the CON group. *Coliform* bacterial population was significantly higher in the AC fed group compared to the CON group, and that in the AR fed group tended to be elevated compared to the CON group.

Discussion

In the present study, we examined the effects of some bovine proteins on serum lipids, liver lipids, faecal lipids, caecal lipids, bacterial population in the colon, and hepatic mRNAs in rats. The serum total cholesterol and non-HDL cholesterol concentration in the AR fed group were significantly lower than those in the CON group at the end of the 4 week feeding period. Several mechanisms have caused to these findings of bovine proteins. One possible reason may be bovine proteins act as dietary fibres. This could be supported by the lower digestibility of AC and AR (92-93%) compared to CON (96%). Jorgensen *et al*. (2003) has reported that increased intake of dietary fibre was associated with a concomitant loss of protein and energy to faeces. Previous findings have showed that dietary fibres lower plasma cholesterol levels in animals and humans compared with casein-based diets (Khosla *et al*.1991; Anderson *et al*.1995). The factor lowering the cholesterol concentration in the AR fed group was the lowering of non-HDL cholesterol level, which was in agreement with previous findings (Fukushima *et al*.2000; Li *et al*.2004). However, there was no significant difference in

hepatic LDL receptor mRNA level in rats fed bovine proteins compared to CON group. Thus, the lower non-HDL cholesterol concentration in the AR fed group may be due to any other reason. This result was in agreement with previous findings showing that there was no correlation between plasma LDL cholesterol concentration and hepatic LDL receptor mRNA level (Sorci-Thomas *et al*.1989). The CYP7A1 mRNA level and feacal bile acid concentration were not significantly different among the group. The rate-limiting enzyme in endogenous sterol biosynthesis is HMG-CoA reductase, which catalyzes the synthesis of mevalonate, and the activity of HMG-CoA reductase is also regulated by changes in the exogenous cholesterol concentration (Brown & Goldstein, 1997). In this experiment, HMG-CoA reductase mRNA expression was significantly lower in AR fed group, and tended to be lower in AC fed group compared to the CON group. This may be another reason for low total serum cholesterol level which was positively correlated with HMG -CoA reductase mRNA level in AR fed group, the correlation coefficient being 0.742 ($P<0.01$). The decrease in HMG-CoA reductase mRNA is surprising and conflicts with previous reports related to dietary fibre. Lund *et al*. (1993) and Moundras *et al*.(1997) have reported that dietary fibre increases hepatic reductase activity. The mechanism of reduction of HMG CoA reducatse activity might be due to the increased liver cholesterol concentration which may inhibit the hepatic HMG-CoA reductase mRNA level. In rats, most of the serum cholesterol (60-80%) is transported in HDL and only 5-10% in LDL, since they are lack of lipid transfer protein (Day *et al*.1979). In contrast, this study showed only 30% of serum cholesterol was from HDL cholesterol even in rats fed the 0.01% and 0.006% bovine dietary cholesterol diets. The dietary cholesterol level and 7% soybean oil content with 0.4% plant sterols in AIN 93G diet may be a reason for lower HDL cholesterol concentration in AC and AR diets fed groups. However, it has been shown that

feeding normal Wistar-Furth or Sprague-Dawley rats with diets containing 1% cholesterol had negiligible effects on serum cholesterol level (Ness *et al*.2004).

Amino acid composition of animal protein might be an important factor affecting behaviour of animal protein. Undigestible behaviour of animal protein may be due to the abound collagen concentration as AC and AR contain abound glycine and proline concentrations (Schubert *et al*.1974). In addition, it has been suggested that favourable amino acid composition is at least partially responsible for the suppressive effect on plasma cholesterol in previous studies (Kayashita *et al*. (1995, 1997), Morita *et al*.1997, Tomotake *et al*.2000, Gudbrandsen *et al*.2005). Lower methionine level or lower methioinine: glycine (Table 2) ratio (80-85%) in AC and AR diets might have hindered the transfer of cholesterol from liver in to blood stream (Morita *et al*.1997). In fact, the lower methionine level may reduce phosphatidyl choline synthesis via phosphatidyl ethanolamine, leading to depression of apolipoprotein release into circulation (Morita *et al*.1997). Moreover, dietary arginine concentration was comparatively higher in AC and AR diets, which was negatively correlated with serum non- HDL cholesterol level($r= 0.863$; $P<0.01$) in a previous study (Eklund & Sjoblom, 1980). These facts may suggest another mechanism of serum cholesterol lowering in AR fed group. On the other hand, effect of methionine would give a reason for higher liver cholesterol level in both AC and AR fed groups. The liver cholesterol concentration was significantly higher in AC fed group, and tended to be higher in AR fed group, than that in the CON group. Han *et al*. (2003) also has reported that liver cholesterol concentration in the groups fed the enzyme resistant fractions of beans was significantly higher than in the cellulose powder fed group, although the reason for this remained unclear, it may be speculated that the increase in liver cholesterol concentration was due to enhanced level of hepatic SR-B1 mRNA level. However, in this study no

significant difference was observed in SR-B1 mRNA level among groups. This finding doesn't agree with previous findings in mice showing that dietary fibres lower liver lipid concentration (Van Bennekum *et al*.2005). However, the significantly higher liver cholesterol level in AC fed group may be a compensatory response to the higher sterol excretion compared to control.

The faecal cholesterol and coprostanol concentration in AC and AR groups were significantly higher than those in the CON group. Neutral sterols occupy a major part of steroid excretion, and it is noteworthy that the excretions of cholesterol and coprostanol were brought by feeding AC and AR. This may be another reason for lower serum cholesterol concentration in AR fed group. This was in agreement with findings for some dietary plant fibres (Kritchesvsky 1988; Eastwood 1992; Moundras *et al*. 1997). Although, the excretion of neutral sterol was affected by bovine protein, acid sterols was unaffected compared with casein. This was in contrast with previous findings showing that some plant fibres lower serum cholesterol by increasing cholesterol elimination as bile acids and neutral sterols (Fukushima *et al.* 2000; Buhman *et al.* 1998). Furthermore, Hara *et al.* (1998) reported that sugar-beet fibre reduces serum cholesterol level by reducing cholesterol synthesis in rats.

According to the results of dietary cholesterol intake, feacal sterol excretion and HMG-CoA reductase mRNA level, the sterol balance was 1.86 folds higher in AC fed group, and that in AR fed group was not different, compared to the control group. Whereas, HMG-CoA reductase mRNA level in AC fed group was not significantly different compared to the control group and that in AR fed group was significantly lower relative to the control. In this study, AC and AR diets may maintain the body sterol balance by two different mechanisms.

Serum TG concentration was significantly lower in the AC and AR fed groups

compared to the CON group. This was in agreement with previous findings showing that dietary fibres reduce serum triglyceride levels in rats (Li *et al.* 2004). Low TG level in AC and AR fed groups was accompanied by the reduction in FAS mRNA level in animal protein fed groups. In fact, the mRNA expression of FAS was significantly lower in AR fed group, and tended to be lower in AC fed group compared to the CON group. The FAS is a multinant enzyme complex that catalyzes the synthesis of long-chain fatty acids from acetyl CoA and malonyl CoA (Wakil *et al.*1983). Moreover, SREBP-1c mRNA level was significantly lower in AR fed group, and it tended to be lower in AC fed group compared to the CON group. The correlation between SREBP-1c and FAS was r =0.660 ($P < 0.01$). It was suggested that the bovine protein-dependant decrease in FAS gene expression is due to the suppression of the gene expression of SREBP-1c, which is the transcriptional factor to generate FAS mRNA. SREBPs are membrane-bound transcriptional factors which regulate the gene expression of enzymes in fatty acid and cholesterol biosynthesis (Brown & Goldstein, 1997; Horton & Shinomura, 1999). SREBP-1c preferentially enhances transcription of genes required for fatty acid synthesis (Horton *et al*.2002).

According to results of this study, anaerobe bacteria population of caecum in the AC and AR fed groups was significantly higher than the CON group. These results were accompanied by higher feacal coprostanol concentration which may be an index of the activity of intestinal flora (Arjmandi *et al*.1992). It was suggested that protein was one of the major substrates for caecal fermentation in rats when animal protein based diet was fed (Tsukahara & Ushida, 2000). Fatty acids of anaerobic bacteria are a major source of energy for the colonic mucosa in humans (Roediger, 1980). *Bifidobacterium* population was significantly higher in the AR fed group,and tended to be higher in the AC fed group compared to the CON group. In addition, coliform

bacteria population was significantly higher in AC fed group and tended to be higher in AR fed group than that in the CON group. However, there was no significant difference in caecal pool size of SCFA among the groups. The reason for higher bifidobacteria and coliform population in bovine protein fed groups remains unclear. However, the undigested AC and AR proteins might be used as nitrogen sources by ceacal bacteria.

In conclusion, effects of some bovine proteins were most clearly seen when compared with rats fed casein. Bovine artery protein (AR) reduced the total serum cholesterol by reducing non-HDL cholesterol, accompanied by lower HMG CoA reductase mRNA level, and by increasing faecal neutral sterol excretion. Animal proteins such as bovine Achilles' tendon protein and artery protein, reduced TG concentration by reducing the fatty acid synthesis in liver accompanied by lower FAS mRNA level compared to the casein fed group. In view some of these facts; we conclude that some bovine animal proteins show dietary fibre like properties by virtue of their low digestibility.

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Abbreviations

AC, Bovine Achilles`tendon protein diet; AR, Bovine artery protein diet; CON, casein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IDL, intermediate density lipoprotein; RT-PCR, reverse transcription polymerase reaction; SCFA, short-chain fatty acid; LDL-R, low density lipoprotein receptor, SR-B1, scavenger receptor class type 1;SREBP-1c,sterol regulatory element binding protein-1c; FAS, fatty acid synthase; cholesterol 7α-hydroxylase (CYP7A1);HMG-CoA reductase, hydroxyl methyl glutaryl-CoA reductase; TG, triglyceride.

Figure legends

Fig. 1. Liver cholesterol concentration in rats fed animal proteins for 4 weeks. Values are expressed as means ± SD for five rats. Values with different superscript letters are significantly different ($P<0.05$).

Figure. 2. Hepatic HMG-CoA reeducate mRNA and cholesterol 7α hydroxylase mRNA expressions in rats fed animal proteins for 4 weeks. Values are expressed as means ± SD for five rats. Mean values with different superscript letters are significantly different ($P<0.05$).

Figure. 3. Hepatic SREBP-1c and fatty acid synthase mRNA expressions in rats fed animal proteins for 4 weeks. Values are expressed as means ± SD for five rats. Mean values with different superscript letters are significantly different ($P<0.05$).

TABLE 1

Composition of experimental diets

Ingredients	Dietary group[1]		
	CON	AC	AR
		g/kg diet	
Casein	200	150	150
Bovine Achilles' tendon protein (cholesterol)	-	50(0.1)	-
Bovine artery protein (cholesterol)	-	-	50(0.06)
Soybean oil	70	70	70
Mineral mixture[2]	35	35	35
Vitamin mixture[3]	10	10	10
Cellulose powder	50	50	50
Sucrose	100	100	100
L-cystine	3	3	3
Choline hydrogen tartrate	2.5	2.5	2.5
3–Butylhydroquinone	0.014	0.014	0.014
α–Corn starch	529.486	529.486	529.486

[1]CON, basal diet; AC, bovine Achilles' tendon diet; AR, bovine artery diet

[2]AIN-93G mineral mixture

[3]AIN-93G vitamin mixture

TABLE 2
Amino acid compositions of bovine proteins

Amino acids (%W/W)	CON	AC	AR
		mmol/g N	
Asp	6.75	6.78	4.85
Thr	4.43	2.69	2.82
Ser	6.24	4.79	3.63
Glu	18.42	10.72	7.57
Gly	3.04	33.30	30.04
Cys	0.26	0.14	0.49
Val	7.31	3.43	10.27
Met	2.48	0.76	0.56
Ile	5.32	2.08	3.67
Leu	9.13	4.69	8.20
Tyr	3.93	0.95	2.60
Phe	3.88	2.17	3.61
Lys	6.92	3.21	2.95
His	2.48	0.92	0.88
Arg	2.70	7.14	3.75
Ala	4.32	-	-
Pro	12.39	16.24	14.09

CON,Casein;AC, bovine Achilles' tendon powder; AR, bovine artery powder

TABLE 3

Serum total cholesterol, VLDL+IDL+LDL-cholesterol, HDL-cholesterol, and triglyceride concentrations in rats fed bovine proteins for 4 weeks[1]

Dietary group	Wk0	Wk2	Wk4
Total cholesterol		*mmol/L*	
CON	2.51± 0.15	2.53± 0.26[a]	2.29± 0.209
AC	2.37± 0.42	2.16± 0.34[ab]	1.99± 0.433
AR	2.46± 0.14	2.08± 0.30[b]	1.94± 0.248 *
HDL-cholesterol			
CON	0.77± 0.02	0.72± 0.10	0.69± 0.08
AC	0.75± 0.15	0.68± 0.13	0.66± 0.14
AR	0.76± 0.05	0.64± 0.10	0.64± 0.08
VLDL+IDL+LDL -cholesterol			
CON	1.75± 0.13	1.81± 0.18[a]	1.59± 0.16
AC	1.62± 0.29	1.47± 0.25[b]	1.33± 0.31
AR	1.70± 0.14	1.45± 0.24[b]	1.31± 0.19 *
Triglyceride			
CON	0.42± 0.15	0.85± 0.24[a]	0.76± 0.17[a]
AC	0.34± 0.16	0.44± 0.12[b]	0.35± 0.16[b]
AR	0.39± 0.07	0.52± 0.08[b]	0.37± 0.16[b]

[1]Values are expressed as means ± SD for five rats. *$P < 0.05$ vs. BD by student's *t*-test.

[a,b]Mean values within a row with unlike superscript letters were significantly different by Duncan's multiple range test ($P < 0.05$).

TABLE 4

Cholesterol intake, faecal neutral steroid and acidic steroid concentrations in rats fed bovine proteins for 4 weeks[1]

	Dietary group		
	CON	AC	AR
		μmol/rat/day	
Cholesterol Intake	-	7.20±1.03	6.91±0.61
Faecal Cholesterol	11.62±4.00[c]	20.69±5.45[a]	12.65±1.72[b]
Faecal Coprostanol	2.20±0.90[c]	12.69±2.50[a]	7.88±2.05[b]
Faecal LCA	0.14±0.07	0.13±0.01	0.12±0.03
Faecal DCA	0.05±0.03	0.07±0.02	0.05±0.03
Faecal CDCA	0.02±0.03	0.03±0.01	0.02±0.02
Faecal CA	0.20±0.11	0.16±0.20	0.08±0.02
Faecal TBA	0.41±0.23	0.39±0.10	0.27±0.04

[1]Values are expressed as means ± SD for five rats. [a,b,c]Means within a row with unlike superscript letters were significantly different ($p<0.05$). LCA, lithocholic acid; DCA, deoxycholic acid; CDCA, chenodeoxycholic acid; CA, cholic acid; TBA, total bile acid.

TABLE 5

Caecal bacterial population in rats fed bovine proteins for 4 weeks[1]

Dietary group	CON	AC	AR
	log10 cfu/g wet caecum		
Bifidobacterium	5.97±0.83[b]	6.30±0.49[ab]	6.65±0.32[a]
Lactobacillus	5.73±0.64[a]	6.12±0.61[a]	5.67±0.83[a]
Coliform	3.91±0.35[b]	4.91±1.36[a]	4.61±0.79[ab]
Anaerobe	6.84±0.33[b]	7.48±0.35[a]	7.31±0.27[a]

[1]Values are expressed as means±SD for five rats. [a,b]Means within the same rows bearing different superscript roman letters were significantly different ($p<0.05$).

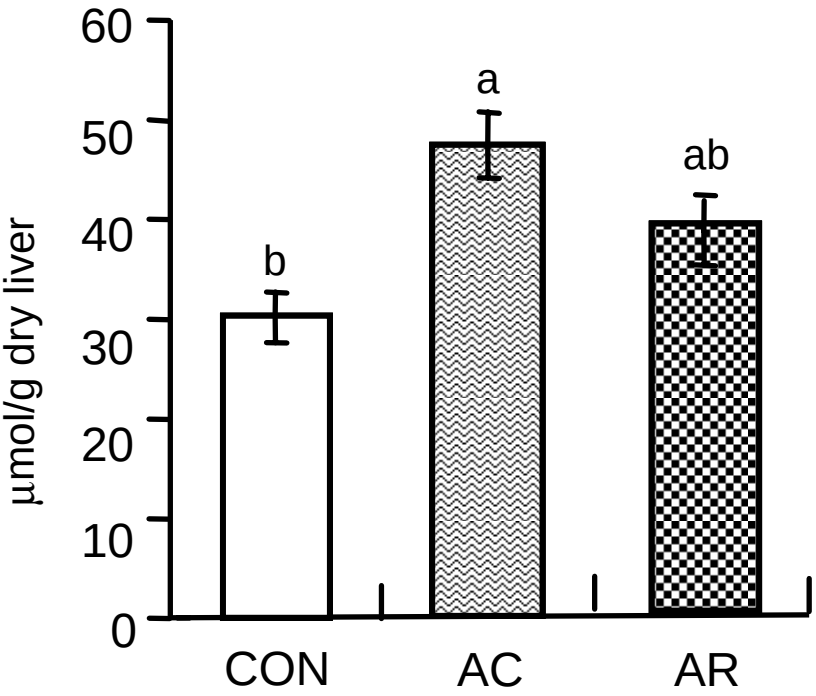

Figure:1 Liyanage Ruvini

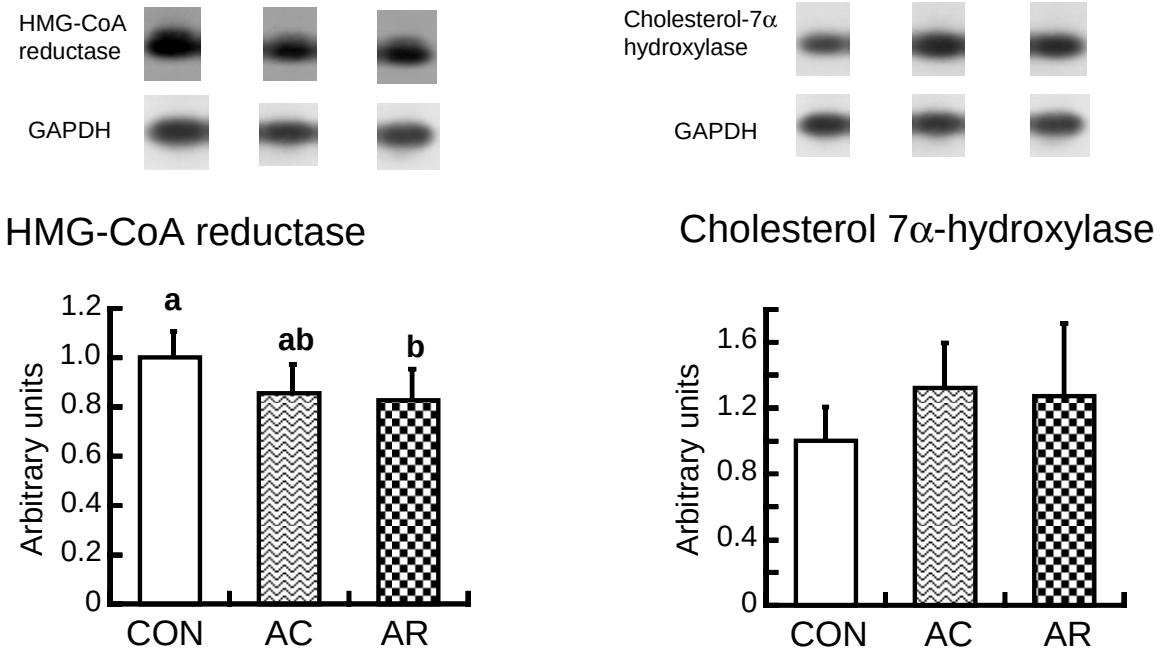

Figure:2 Liyanage Ruvini

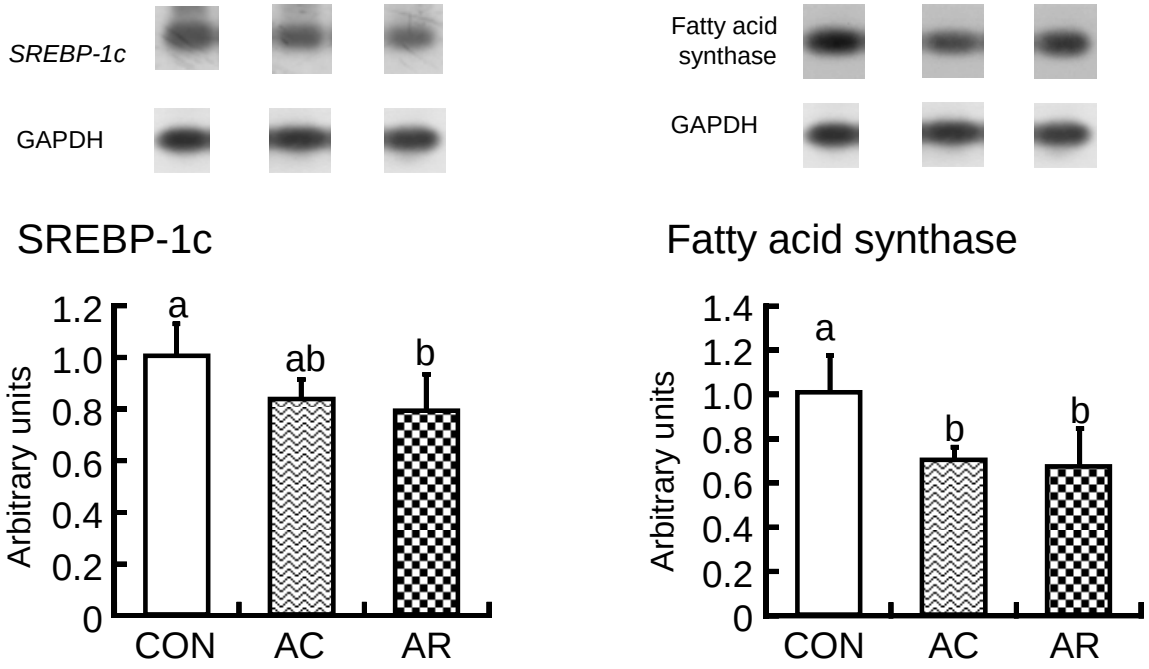

Figure:3 Liyanage Ruvini