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Supplementation of Probiotics on Feed Intake, Digestibility and Conjugated Linoleic Acid Contents in Plasma and Meat of Growing Goats

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Abstract: This experiment was performed with the purpose of investigating effect of additional blend of Saccharomyces cerevisiae and Lactobacillus acidophilus probiotics on growth, ruminal metabolism and plasma fatty acid profiles particularly Conjugated Linoleic Acid (CLA) in growing goats fed corn silage and selected the optimal levels of the probiotics for further study. Twenty-four growing crossbred (Thai native x Anglo-Nubian) goats that weighed (14.2±2.3 kg), aged about 6 months, were purchased and allocated to 4 treatments according to Randomized Complete Block Design (RCBD) with 6 goats in each treatment. The blocks were made by weight into heavy, medium and light goats and each of the treatments contained two goats from each of the blocks. The results displayed that g kg-1 W0.75 dry matter intake (p<0.05), average daily gain (g day-1) (p<0.01) and feed conversion (p<0.05) were increased. At the same time digestibility of neutral detergent fiber (p<0.05), ether extract, acid detergent fiber and CP (p>0.05) as well as that of dry matter and organic matter (p>0.05) also were increased. In the mean time, ruminal average pH unaffected, but the NH₃-N and also plasma urea nitrogen (p<0.05), total volatile fatty acid (p>0.05) were raised, but propionic proportion (p<0.05) and butyric proportion (p>0.05) were reduced in concurrent with raise of acetic proportion and resultantly C2:C3 ratio (p>0.05). Protozoa number (p>0.05) was depressed contrasted to heighten total viable bacterial number. On plasma fatty acid profiles, total saturated fatty acids (p>0.05) was increased and contrasted with decrease of C15:0 (p<0.01), C16:0 (p>0.05) and C18-C22 polyunsaturated fatty acids (p<0.05 or p<0.01). In addition, the experiment proved that the supplemented probiotics was in force for heightening CLA (p<0.01); for raising desirable fatty acids (p<0.05); for reducing ratio of PUFA: SFA (p>0.05) and for raising ratio of n6:n3 (p<0.05).

Key words: Probiotic, conjugated linoleic acid, carcass, digestibility, plasma fatty acid, goats

INTRODUCTION

Now-a-days, public requirements for food quality and safety, environmental deterioration and pollution, together with animal welfare have become the keystone that should be considered in animal agriculture. It is obvious that the corn silage is appropriate to be employed in intensive and extensive goat industry for it can be free from the seasonal limitation and suitable for mechanization or highly-technological feeding.

A probiotics was defined as a living single or mixed microbial which beneficially affects the host animal by improving its gastrointestinal microbial balance (Krehbiel *et al.*, 2003). Despite the fact that there is no probiotics can compete antibiotics with functions of growth stimulating and prevention or treatment of diseases, but as a nuisance free feed additive, they are widely embroiled in *in vitro* or *in vivo* studies. In summation, the utilization of probiotics have mainly

regarded the administration of yeast cultures partially strains of S. cerevisiae (Chaucheyras and Fonty, 2001). Moreover, in parallelism yeast, Lactobacilli have drawn much study interest by the reason of providing the host animal healthier and more favorable gastro-enteric setting for digestive and absorption processes (Klaenhammer, 1998). There were abundant literatures to prove that among several Lactobacilli strains (L. acidophilus, L. casei and L. bifidus), L. acidophilus was surely the most focalized one on productive performances, on the variation of intestinal flora and on the sanitary state of the host animals (Krause et al., 1995; Tannock et al., 1990). The studies related to effect of L. acidophilus on rumen fermentation and animal production (i.e., body gain, feed efficiency, milk yield and quality and meat quality) are scarce. Lots of research demonstrated that L. acidophilus in combination with fungal cultures was more efficacious for increasing milk production in lactating dairy cows. Furthermore, found

the *L. acidophilus* resistant to pH 2.0 and bile salts (0.3%) and could be pre-selected as a probiotics for use in goat feed

Conjugated Linoleic Acid (CLA) is a collective term used to describe one or more positional and geometric isomers of linoleic acid with conjugated double bonds (Ip et al., 1994) and it is characterized as two double bonds separated by a signal bone at various carbon positions. CLA has been reported for wide range of effects such as anticarcinogenic, antiatherogenic, antidiabetic and immune stimulatory. They have also been shown to alter partitioning and lipid metabolism and reduce body fat in a number of different animal species (Bauman et al., 2000). Ruminant products are the predominant CLA resource. Whereby, it is fantabulous and interesting to work at enhancing of CLA concentration in ruminant products with the aim to meet the effectual level for human being. The present experiment was carried out to study the effect of additional S. cerevisiae and L. acidophilus probiotics on growth, ruminal metabolism and plasma fatty acid profiles particularly CLA in growing goats fed with corn silage and selected the optimal levels of the probiotics for further study.

MATERIALS AND METHODS

Animals and management: Twenty-four growing crossbred (Thai native x Anglo-Nubian) goats that weighed 14.2±2.3 kg, aged about 6 months, were purchased from Pukthongchai district, Nakhon Ratchasima province of Thailand to perform this experiment. The animals were allocated to 4 treatments according to Randomized Complete Block Design (RCBD) with six goats in each treatment. The blocks were made by weight into heavy, medium and light goats and each of the treatments contained two goats from each of the blocks (Table 1). Before experiment, the animals were injected with Ivomic (Merial Ltd., Iselin, NJ) for anti-internal parasite and housed in individual pens (0.9×1.4 m) where the animals could have an easy access to corn silage and fresh water ad libitum. What was more, the pens were cleaned and disinfected with Ciber solution prior to the housing of the animals. During the experiment, animals in different treatments received the whole plant corn silage plus concentrate basal diet and supplemented with 0, 2.5, 5 and 7.5 g/h/day probiotics (L. acidophilus

Table 1: Lay-out of experimental treatments

Groups	Animals (n)	BW (kg)	Treatments
I (Control)	6	14.03 ± 2.4	Basal diet + probiotics 0 g day ⁻¹
II	6	14.87±2.9	Basal diet + probiotics 2.5 g day ⁻¹
Ш	6	13.93 ± 2.5	Basal diet + probiotics 5.0 g day ⁻¹
IV	6	14.05±2.4	Basal diet + probiotics 5.0 g day ⁻¹

Basal diet = Whole plant com silage plus concentrate

about 2.0×10^{12} cfu g⁻¹ and *S. cerevisiae* about 5.0×10^{11} cfu g⁻¹). The additional probiotics was mixed evenly with concentrate prior to feeding and offered to animals by half at 9:00 am and the other at 3:00 pm, respectively. The concentrate was supplied by 1.5% percentage on body weight for each goat to ensure that the dietary intakes of crude protein, growth net energy and dry matter in accordance with the Nutrients Requirements of Goats (1989) under the condition of maintenance plus lower activity and 50 g day⁻¹ weight gain. All animals accessed to the whole plant corn silage and clean water *ad libitum*. The experiment lasted 8 weeks, excepting 2 weeks for adjustment, 1 week for adaptation and 1 week post-experiment for urinary and faecal samples collection.

Experimental material: The probiotics was purchased from L. P. Feeds Tech Co., Ltd (Bangkok, Thailand), containing L. acidophilus about 2.0×10^{12} cfu g⁻¹ and S. cerevisia about 5.0×10^{11} cfu g⁻¹. The whole plant corn silage was purchased from Kornburee Cooperatives (Kornburee district, Nakhon Ratchasima province of Thailand). The pelleted concentrate was supplied by the farm of Suranaree University of Technology (Nakhon Ratchasima province of Thailand) and it was composed of cassava chip (12.0%), cassava pulp (31.5%), rice bran with germ (10.0%), defatted rice bran (10.0%), molasses (8.0%), palm kernel expeller meal (18.0%), rapeseed meal (4.0%), corn meal (4.0%), urea (1.8%), mineral (1.5%) (Containing Ca 14.5%, P 17%, NaCl 18%, Mg 10% and carrier) and additional binder (0.2%).

Sampling: The daily offered and left concentrate and whole plant corn silage were weighed (the residues were removed) every morning before offering for the purpose of determination dry matter intake. Body weight of the animals were measured weekly prior to the morning feeding with the aim of evaluating the growing performances. The whole plant corn silage and concentrate were sampled weekly and dried at 60~65°C in hot air oven for determination of Dry Matter (DM) composition and followed by grounding through a 1 mm screen and then kept in tightly covered plastic containers to make a pool respectively for further proximate analysis. During the post-experiment week for urinary and faecal samples total collection, the all-day faeces and urine (10% H₂SO₄ was used as a preserving reagent, 30 mL/container) were collected and the total amount was recorded down every morning (measured faeces weight and urine volume). Subsequently, 15% of the total amounts was sub-sampled to make a pool respectively for each animal and then was kept at -20°C and in the end was dried prior to chemical composition analysis that aimed to determine digestibility and nitrogen balance. For ruminal fluid samples, they were withdrawn on the last day of the experiment through an esophageal stomach tube following 0, 3 and 6 h post-morning meal timing. The samples were strained through three layers of muslin cloth and then were followed by immediately measuring of pH with an OHS-3C pH meter. Thereafter, 1 mL of the samples were measured well and truly with a pipette into the tubes containing 9 mL 10% formalin (V: V = 9:1) as a preserving reagent and then were closed tightly with screw caps that with butyl rubber lining for checking the counts of ruminal protozoa and bacteria.

At the same time, 20 mL of the samples were measured and then put into small plastic bottles containing 5 mL 6 N HCl as a preserving reagent and then the bottles were closed tightly with screw caps that with butyl rubber lining for determination of ruminal ammonia N and volatile fatty acids. With that, all samples were kept at -20°C, until further analysis. The blood samples were collected from jugular veins into EDTA containing vacuum tubes and were centrifuged at 2700× r for 5 min to separate plasma from the cells within 20 min after sampling. Subsequently the plasma was collected and then it was stored at -80°C for subsequent analyses of blood urea nitrogen and fatty acid profiles.

Chemical analysis and calculation: The Dry Matter (DM) of feed (including residue) and feces samples were determined in triplicate by drying in a hot air oven at 60~65°C for 48 h and the Organic Matter (OM), N (feed N, faecal N and urinary N) and crude ash were determined according to the methods described in AOAC (1990). The Neutral Detergent Fiber (NDF) compositions were determined by the method stated by Van Soest *et al.* (1991).

An OHS-3C pH meter was used to measure the ruminal pH and the counts of ruminal protozoa and bacteria were directly checked on a Tiefe Depth Profondeur by an electron microscope under 40-fold directly. The determination of apparent digestibility and nitrogen balance was done.

The ruminal fluid samples that used to determine total VFA and molar proportion of main VFA mix (acetate, propionate and butyrate) were centrifuged at 3500× r for 10 min at 4°C to get rid of food particles and ruminal microbe, with that measured 1 mL supernatant into a 2 mL vial for Gas Chromatography (GC) analysis. The preparation of plasma samples for GC analysis was done by using a modified method explained by Bondia-Pons *et al.* (2007).

Analysis of fatty acids by Gas Chromatography (GC): Total VFA and molar proportion of acetic, propionic and butyric acids in ruminal fluid and fatty acid profile of plasma samples were determined by HP6890 Gas

Chromatography (GC) (made in USA) that fitted with a Flame Ionization Detector (FID). In addition, a J and W 122~3232 column was applied for determination of VFA, whereas a 100 m ×0.25 mm fused silica capillary column (SP2560, Supelco Inc, Bellefonte, PA, USA) for determination the plasma fatty acid profiles. The column temperature was fixed at 70°C for 4 min, then it increased at 13°C min⁻¹ to 175°C which lasted for 27 min. Continually it increased at 4°C min⁻¹ to 215°C and kept for 31 min. Nitrogen was adopted as carrier gas with a 60 mL min⁻¹ flow rate and the oven temperature was 250°C. FID and injection temperature were fixed at 280°C and a 1 μL injection was done with a 10 μL injector.

Data analysis: Data were analyzed according a randomized complete block design. Variation due to blocks was extracted in the models employed for the analysis. The protected least significant differences method was used to determine differences among treatment means. Polynomial contrasts (linear, quadratic and cubic effects) were used to evaluate the all effects. In addition, a non-parametric Mann-Whitney test was used to compare the count means of rumen protozoa also viable bacteria within groups. Differences were considered to be significant at p<0.05 (*), highly significant at p<0.01 (**), tendencies at 0.05<p>0.050 and 'ns' was used to represent no significant difference.

RESULTS

All animals received a diet composing of whole plant silage plus concentrate. The diet was adequate to meet the requirements of crude protein, growth net energy and dry matter intakes of the goats under the condition of maintenance plus lower activity and 50 g day⁻¹ weight gain Nutrients Requirements of Goats (1989). As to the concentrate, it contained DM 88.8%, CP 13.4% and NDF 37.1%, whereas the silage contained DM 21.9%, CP 9.2% and NDF 57.9% (DM basis) (Table 2). As shown

Table 2: Chemical compositions of experimental diet (dry matter basis)

Items	Composition (%)
Concentrate	
Dry matter	88.8
Organic matter	93.4
Crude protein	13.4
Ether extracts	4.0
Acid insoluble ash	3.8
Acid detergent fiber	28.7
Neutral detergent fiber	37.1
Corn silage	
Dry matter	21.9
Organic matter	88.1
Crude protein	9.2
Ether extract	1.8
Acid insoluble ash	6.1
Acid detergent fiber	46.6
Neutral detergent fiber	57.9

in Table 3, the main fatty acids of the concentrate were comprised of 30.72% C18:2n6c, 20.0% C17:0, 15.34% C12:0, 14.75% C18:1n9c. Concededly, these fatty acids accounted for 1.23, 0.80, 0.62 and 0.59% of the concentrate dry matter respectively. And yet, the main fatty acids of the whole plant corn silage were composed of 39.10% C18:2n6c, 16.60% C18:1n9c, 14.90% C16:0 and 11.71% C18:3n3 and these fatty acid mad up of 0.70, 0.30, 0.27 and 0.21% of the corn silage dry matter respectively.

No differences existed in whole plant corn silage and concentrate total daily average as well as centesimal body weight dry matter intakes between the treatments. However, as obviously shown in Table 4, in terms of $g kg^{-1} W^{0.75}$ Total Dry Matter Intakes (TDMI) significantly increased with linear, quadratic, also cubic statistical analysis brought on addition of probiotics. Wherein, the increasing levels of supplemented probiotics did not bring out any differences in impacts of probiotics on dry matter intakes. On the contrary, the increasing levels (2.5, 5.0, 7.5 g/h/day) showed similar g kg⁻¹ W^{0.75} TDMI (52.3, 52.1, 52.5). When judging the growth performance with ADG, the linear, quadratic together with cubic statistical analysis showed that it increased with extremely significant differences. Whereas, the comparisons of the ADG within probiotics supplemented

Table 3: Fatty acid profiles of concentrate and whole plant core silage (DM basis)

Items	DM (%)	Total fatty acid (%)
Concentrate		
C12:0	0.62	15.34
C14:0	0.23	5.83
C16:0	0.25	6.19
C17:0	0.80	20.00
C18:0	0.09	2.28
C18:1n9c	0.59	14.75
C18:2n6c	1.23	30.72
C18:3n3	0.07	1.79
Others	0.12	3.00
Corn silage		
C14:0	0.03	1.60
C16:0	0.27	14.90
C16:1	0.01	0.61
C17:0	0.03	1.60
C18:0	0.07	3.68
C18:1n9c	0.30	16.60
C18:2n6c	0.70	39.10
C18:3n3	0.21	11.71
Others	0.18	10.09

groups were quite close to each other regardless of increasing doses (supplemented probiotics 2.5, 5.0, 7.5 g/h/day, the ADG were 52.7, 54.8 and 51.4 g day⁻¹, respectively). In the case of checked growth performance with feed efficiency (DMI: ADG), the linear, quadratic as well as cubic significant difference predicatively occurred not only in comparison with control group, but also in comparison within treatment groups (Table 4). The DMI: ADG ratios of 2.5 and 5.0 g/h/day probiotics treatment groups were close to each other (7.6 and 7.1). Nevertheless, those of 5.0 and 7.5 g/h/day probiotics treatment groups were significantly differed to each other (7.1 and 8.6). For a holoscopic look of Table 3 and 4, it showed that the growth performance of 5.0 g/h/day probiotics reached highest ADG and feed efficiency $(54.8g \text{ day}^{-1} \text{ and } 7.1) \text{ compared with dose of } 2.5 (52.7)$ g d and 7.6) and 7.5 g/h/day (51.4 g day⁻¹ and 8.3).

The growth performance was detected by weekly gain. The first weekly gain of control group reduced due to outset of experiment, contrasted with it, probiotics treatment groups showed more steady weekly gain. In the last second week of this experiment, the hot weather (34±3.8°C) stressed the animals and all of them reduced intake owing to the weather change. As a result, the weekly gain promptly decreased, whereas, the probiotics treatment groups turned back to stable gain in the coming week, contrariwise, the control group continued lowering. These evidences testified to the efficiencies of probiotics on adaptation of the animals to feed and heat stress.

Table 5 showed the digestibility of DM, OM, CP, ADF and EE were not significantly different because of additional probiotics. Thereunto, the digestibility of EE, ADF and CP were in the line with expectation to show increasing tendency (p>0.05). At the meantime, the supplementation of probiotics was effectual to elevate the digestibility of NDF with difference in linear, quadratic also cubic significant.

Supplementation of probiotics did not conduce to significant changes for the ruminal average pH, howbeit the 5.0 g/h/day group was observed a decreasing tendency comparing to the control (6.42 vs. 6.72) (p>0.05) (Table 6). Differed from the case of pH, ammonia nitrogen

Table 4: The effect of probiotics on DMI, ADG and feed conversion of growing goats

	Probiotics (Probiotics (g/h/day) Contrast						
Treatment groups	0	2.5	5.0	7.5	SEM	Linear	Quadratic	Cubic
SDMI (g day ⁻¹)	176.0	173.6	161.9	199.7	10.51	NS	NS	NS
CDMI (g day ⁻¹)	227.5	227.5	227.5	227.5	1.02	NS	NS	NS
Total (g day ⁻¹)	403.5	401.1	389.4	427.2	17.53	NS	NS	NS
g kg ⁻¹ W ^{0.75}	48.4 ^b	52.3ª	52.1ª	52.5ª	1.10	*	*	*
Body weight (%)	2.4	2.7	2.7	2.8	0.12	NS	NS	NS
ADG (g day ⁻¹)	$41.1^{\rm b}$	52.7ª	54.8°	51.4ª	1.30	***	aje aje	**
DMI: ADG	9.8⁴	7.6 ^{bc}	7.1°	8.3 ^b	1.32	*	*	*

SDMI = Whole plant corn Silage Dry Matter Intake; CDMI = Concentrate Dry Matter Intake; LWI = Live Body Weight Intake (%); Means with different superscript letters in the same row differ significantly (p<0.05); SEM = Standard Error of the Mean; *p<0.05; **p<0.01; NS = Not Significantly different (p>0.05)

Table 5: The effect of probiotics on dietary digestibility of growing goats fed whole plant corn silage (%)

	Probiotics ((g/h/day)				Contrast		
Treatment								
groups	0	2.5	5	7.5	SEM	Linear	Quadratic	Cubic
DDM	69.1	69.9	72.2	69.2	0.88	NS	NS	NS
DOM	73.5	74.6	75.7	74.0	0.85	NS	NS	NS
DCP	61.7	63.3	65.4	64.3	0.95	NS	NS	NS
DADF	39.2	42.7	42.9	39.8	0.87	NS	NS	NS
DNDF	52.8 ^b	56.7ª	57.2ª	53.2 ^b	0.71	*	*	*
DEE	75.0	76.4	78.0	75.1	1.05	NS	NS	NS

Means with different superscript letters in the same row differ significantly (p<0.05); SEM = Standard Error of the Mean; *p<0.05; **p<0.01; NS = Not Significantly different (p>0.05)

Table 6: The effect of probiotics on the average pH, ammonia nitrogen (NH₃-N, mg DL⁻¹), plasma nitrogen (PUN, mg DL⁻¹) and VFA (mM L⁻¹) of growing goats fed whole plant corn silage

	Probiotics	(g/h/day)				Contrast			
Treatment groups	0	2.5	5.0	7.5	SEM	Linear	Quadratic	Cubic	
pH	6.72	6.63	6.42	6.58	0.06	NS	NS	NS	
NH ₃ -N	10.43 ^b	12.51ª	12.32^{a}	12.14ª	0.27	*	*	*	
PUN	11.01 ^b	16.31ª	16.48⁴	15.88a	0.34	*	*	*	
TVFA	56.22	56.82	56.93	59.28	0.70	NS	NS	NS	
VFA proportion (TVFA	A%)								
Acetate	66.28 ^b	67.82^{b}	68.37 ⁶	69.23ª	1.09	NS	NS	NS	
Propionate	21.51ª	19.12^{b}	19.47 ⁶	19.68 ^b	0.65	*	*	*	
Butyrate	6.83	5.98	6.12	6.23	0.40	NS	NS	NS	
$C_2:C_3$	3.79°	4.49 ^a	4.42°	4.42ª	0.15	*	*	*	

Means with different superscript letters in the same row differ significantly (p<0.05); SEM = Standard Error of the Mean; *p<0.05; NS = Not Significantly different (p>0.05)

Table 7: The effect of probiotics on rumen microbe population of growing goats fed whole plant com silage

	Probiotics ((g/h/day)			Contrast				
Treatment groups	0.0	2.5	5.0	7.5	SEM	Linear	Quadratic	Cubic	
Protozoal population (×10	p ⁴)								
0 h	0.86	0.68	0.75	0.83	0.09	NS	NS	NS	
3 h	1.18	1.18	1.10	1.13	0.10	NS	NS	NS	
6 h	0.88	0.72	0.73	0.86	0.09	NS	NS	NS	
Bacterial population (×10	¹⁰)								
0 h	1.17	1.27	1.30	1.13	0.08	NS	NS	NS	
3 h	1.78 ^b	2.08^{a}	2.18⁴	2.02ª	0.14	oje oje	9¢ 9¢	oje oje	
<u>6 h</u>	1.48 ^b	1.68a	1.73ª	1.57 ^b	0.08	*	*	*	

Means with different superscript letters in the same row differ significantly (p<0.05); SEM = Standard Error of the Mean; *p<0.05; NS = Not Significantly different (p>0.05); L = Linear; Q = Quadratic; C = Cubic

(NH₃-N) and Plasma Nitrogen (PUN) significantly increased as a causation of supplementing probiotics (p<0.05). In terms of Volatile Fatty Acid (VFA), the total production of VFA was entailed to a faint increment (p>0.05) and butyric centesimal proportion in the round way to show a slight decrement (p>0.05) with increasing levels of probiotics. However, the increasing level of probiotics tended to increase the acetic centesimal proportion and up to a significant amount (p<0.05) in comparison with the control (69.23 vs. 66.28 mM L⁻¹) at the level of 7.5 g/h/day. The propionic centesimal proportion showed linear, quadratic and cubic decrease due to the addition of probiotics (p<0.01), but then it was similar within treatment groups. Regarding to the ratio of C₂: C₃, addition of probiotics affirmatively brought it on linear, quadratic as well as cubic increase comparing to the control (p<0.05) and yet, it was almost the same within the probiotics treatment groups (4.49, 4.42 and 4.42).

The number of protozoa ranged from $0.68\text{-}1.18\times10^4$ mL⁻¹ rumen fluid. In addition, as expected, even though

the effectiveness of supplemented probiotics on protozoa population was not significant (p>0.05), an overt subtraction was found. Particularly 2.5 and 5.0 g/h/day, 2 levels let the counts of protozoa down by a visible tendency (p>0.05) (Table 7). The number of total viable bacteria ranged from 1.17-2.02×10¹⁰ mL⁻¹ rumen fluid. Before morning meal, the addition of probiotics did not open the door for pushing up the counts of total ruminal bacteria by any significance, except for the 2.5 and 5.0 g/h/day, 2 treatments presented a raising tendency (p>0.05). Howbeit the effect of additional probiotics on ruminal bacterial number displayed enhancement with significant or highly significant differences after feeding 3 h (p<0.01) or went to the length of 6 h (p<0.05) with linear, quadratic and cubic statistical analysis (Table 6). The case was congruent with that of protozoa, a line chart could clearly show the elevation of bacterial numbers that were induced by additions of probiotics.

The total dietary N intake and faecal N excretion were not statistically different for all treatments. Yet the urinary

Table 8: The effect of probiotics on nitrogen balance of growing goats fed whole plant corn silage

	Probiotic(g/h/day)				Contrast	Contrast			
Treatment groups	0	2.5	5	7.5	SEM	Linear	Quadratic	Cubic		
N intake (g day ⁻¹)	7.5	7.4	7.3	7.8	1.20	NS	NS	NS		
N excretion (g day ⁻¹)										
Faece	3.3	3.3	3.3	3.4	0.43	NS	NS	NS		
Urine	1.6°	$1.7^{ m ab}$	1.8ab	1.9⁴	0.04	*	NS	NS		
Total	4.9°	5.0 ^b	5.1 ^{ab}	5.3ª	0.31	*	NS	NS		
N absorption (g day ⁻¹)	4.8^{ab}	4. 7⁰	4.6^{b}	5.0ª	0.29	NS	NS	NS		
N retention (g day ⁻¹)	2.6ª	2.4^{ab}	$2.2^{\rm b}$	2.5^{a}	0.33	NS	NS	NS		
N retention (%)	34.7	32.4	30.2	32.1	1.53	NS	NS	NS		

Means with different superscript letters in the same row differ significantly (p<0.05); SEM = Standard Error of the Mean; *p<0.05; NS = Not Significantly different (p>0.05); L = Linear; Q = Quadratic; C = Cubic

Table 9: Plasma fatty acids centesimal profiles of growing goats supplemented probiotics under condition of feeding whole plant corn silage

EA (TEAO()	Probiotics (g/	h/day)		•		Contrast		
FA (TFA%)	0	2.5	5	7.5	SEM	L	O	С
C8:0	0.72ª	0.68ª	0.50°	0.59 ⁶	0.05	*	*	NS
C10:0	$0.15^{\rm b}$	0.29ª	0.26ª	0.22ª	0.03	**	***	**
C12:0	0.38^{b}	0.36bc	0.50°	0.26°	0.04	NS	NS	NS
C14:0	3.31^{b}	3.89ª	3.34^{b}	3.89 ^a	0.15	*	NS	*
C15:0	0.45a	0.39 ^a	0.17 ⁶	0.23 ^b	0.05	**	*	*
C16:0	17.77	16.75	17.59	16.20	0.70	NS	NS	NS
C16:1	0.84	0.88	0.81	0.77	0.08	NS	NS	NS
C17:0	2.92	2.82	2.86	3.10	0.20	NS	NS	NS
C18:0	22.74	23.04	23.15	24.26	1.11	NS	NS	NS
C18:1n9t	1.88	1.87	1.96	1.87	0.06	NS	NS	NS
C18:1n9c	16.60	16.41	17.07	17.58	0.70	NS	NS	NS
C18:2n6c	15.80	15.10	15.44	15.35	0.70	NS	NS	NS
C18:3n3	1.04ª	0.96⁴	0.86 ^b	0.75 ^b	0.05	*	*	*
C18:c9.t11	0.47 ^b	0.60 ^a	0.66ª	0.58°	0.03	*	*	*
C18:t10,c12	0.00^{b}	0.07^{a}	0.08^{a}	0.06^{a}	0.01	**	sk sk	**
C20:2	0.95ª	0.60^{bc}	0.70 ^b	0.52°	0.02	**	sk sk	**
C20:3n3	2.82ª	2.21 ^b	2.37 ^b	2.57°	0.12	*	*	*
C20:3n6	0.30^{a}	0.21 ^b	0.19^{b}	0.24 ^b	0.02	**	**	**
C20:4n6	3.11°	3.94⁴	3.51^{bc}	3.64ab	0.24	*	*	*
C20:5n3	0.41ª	0.35 ^b	0.35 ^b	0.30°	0.01	**	**	**
C24:0	1.16^{a}	0.27°	0.21 ^b	0.29 ^b	0.10	**	**	**
C24:1	2.45	2.54	2.37	2.45	0.04	NS	NS	NS
C22:6n3	3.36	3.13	3.34	3.15	0.18	NS	NS	NS
TSFA	47.60	48.59	48.58	49.04	1.52	NS	NS	NS
TMUSFA	21.77	21.70	22.51	22.67	1.07	NS	NS	NS
TPUSFA	28.26	27.10	27.73	27.14	1.00	NS	NS	NS
DFA	70.76	72.94	73.43	73.77	2.37	NS	NS	NS
PUSFA/TSFA	0.59	0.56	0.57	0.55	0.01	NS	NS	NS
Tn6	19.68	21.05	21.04	19.87	0.90	NS	NS	NS
Tn3	7.63	6.65	6.92	6.77	0.09	NS	NS	NS
n-6/n-3	2.58°	3.05ª	2.91ª	2.94ª	0.18	*	*	*

TSFA = Total Saturated Fatty Acid; TMUSFA = Total Mono-Unsaturated Fatty Acid; TPUSFA = Total Poly-Unsaturated Fatty Acid; DFA = Desirable Fatty Acid; Tn6 = Total n6 fatty acid; Tn3 = Total n3 fatty acid; Means with different superscript letters in the same row differ significantly (p<0.05); SEM = Standard Error of the Mean; *p<0.05; NS = Not Significantly different (p>0.05); L = Linear; Q = Quadratic; C = Cubic

and total N excretions were pushed up with the increasing levels of additional probiotics, thereof raised to linear significant difference for the level of 7.5 g/h/day in comparison with the control (1.0 and 3.8 g day⁻¹ vs. 0.7 and 3.4 g day⁻¹) (p<0.05) (Table 8).

There were not significant effects on the N absorption (g day⁻¹), N retention (g day⁻¹) as well as N retention (%) because of addition of probiotics compared with the control, but then those of the 7.5 g/h/day treatment were significantly higher (p<0.05) than those of 2.5 and 5.0 g/h/day.

To sum up, the effects of supplemented probiotics on the N-balance of growing goats fed whole plant corn silage displayed in enlarging the urinary and accordingly total N excretion. Still, it presented no performances with statistical difference on N absorption also N retention.

Specifically, supplementation of probiotics was with effect on fatty acids centesimal composition of plasma by: pushing up the C10:0 with linear, quadratic also cubic significance (p<0.01); raising C14:0 with linear and cubic significance (p<0.05); declining C16:0 and C17:0 with tendencies but C15:0 with significant difference (linear: p<0.01; quadratic and cubic: p<0.05).

Table 10: Fatty acid and conjugated linoleic acid contents (μg mL⁻¹ plasma) in plasma of growing goats supplemented probiotics under condition of feeding whole plant corn silage

whole plant	corn silage								
	Supplement	ted probiotics (g/h/o	lay)			Contrast	ıst		
FA (μg mL ⁻¹ plasma)	0	2.5	5.0	7.5	SEM	L	Q	C	
C8:0	7.0ª	6.5b	6.9ª	6.8ab	0.31	*	**	*	
C10:0	1.3°	2.6ª	2.6^{a}	1.9°	0.26	oje oje	**	***	
C12:0	$3.4^{\rm b}$	$3.3^{\rm b}$	3.8^{a}	$3.3^{\rm b}$	0.06	*	NS	NS	
C14:0	29.8°	35.2ª	32.6ab	34.0^{a}	0.64	*	**	s ļ c	
C15:0	4.1ª	3.5^{b}	$2.6^{\rm d}$	3.0°	0.25	**	**	s ļ c	
C16:0	160	151.6	151.9	141.7	7.63	*	**	s ļ c	
C16:1	7.6ª	8.0ª	7.9 ^a	6.9^{b}	0.47	*	NS	NS	
C17:0	26.3ab	25.5 ^b	27.9 ^{ab}	28.9ª	0.39	NS	NS	NS	
C18:0	196.8	208.5	226.2	212.2	11.08	NS	NS	NS	
C18:1n9t	16.9	16.9	17.1	16.4	1.03	NS	NS	NS	
C18:1n9c	149.5	148.5	146.8	153.8	3.14	NS	NS	NS	
C18:2n6c	152.3	145.7	150.6	143	3.01	NS	NS	NS	
C18:3n3	9.3ª	8.7ª	8.4^{ab}	6.5 ^b	0.66	*	**	钟	
C18:c9,t11	4.2°	5.4 ^b	6.4ª	5.1 ^b	0.43	*	**	钟	
C18:t10,c12	0.0°	$0.6^{\rm ab}$	0.7ª	0.5 ^b	0.40	**	**	***	
C20:2	8.6ª	5.4°	6.9 ⁶	5.8 ^{oc}	1.08	**	**	***	
C20:3n3	25.4ª	20.0°	23.2ab	22.5bc	0.79	*	**	钟	
C20:3n6	2.7	2.6	2.3	2.1	0.42	*	**	钟	
C20:4n6	28.1 ^b	35.6ª	34.3ª	33.1ª	0.91	*	**	钟	
C20:5n3	3.7ª	2.3°	2.96	2.4°	0.20	**	**	***	
C24:0	10.4^{a}	4.3 ^b	$4.1^{\rm b}$	4.5 ^b	1.07	**	**	***	
C24:1	22.1	23.0	23.2	21.4	0.90	NS	NS	NS	
C22:6n3	30.2ª	$28.4^{ m ab}$	26.9°	27.6°	1.33	3 4	NS	NS	
TSFA	428.6	441.5	458.6	436.3	5.15	NS	NS	NS	
TMUSFA	196.1	196.4	215.0	198.5	2.07	NS	NS	NS	
TPUSFA	264.5	254.9	272.6	258.6	1.02	NS	NS	NS	
DFA	637.3	660.0	717.6	645.4	10.79	3 4	NS	NS	
PUFA/SFA	0.62	0.58	0.59	0.59	0.01	NS	NS	NS	
Tn6	177.3^{b}	190.1ª	204.3ª	183.8 ⁶	5.03	oje	*	NS	
Tn3	68.6ª	59.4 ^b	61.4^{b}	59.0°	0.99	*	NS	NS	
n-6/n-3	2.58^{b}	3.20^{a}	3.33ª	3.12ª	0.07	*	**	słc	

TSFA = Total Saturated Fatty Acid; TMUSFA = Total Mono-Unsaturated Fatty Acid; TPUSFA = Total Poly-Unsaturated Fatty Acid; DFA = Desirable Fatty Acid; Tn6 = Total n6 fatty acid; Tn3 = Total n3 fatty acid; Means with different superscript letters in the same row differ significantly (p<0.05); SEM = Standard Error of the Mean; *p<0.05; NS = Not Significantly different (p>0.05); L = Linear; Q = Quadratic; C = Cubic

In point of impacts on C18 fatty acids centesimal composition of plasma resulted from supplementation of probiotics, the highlight existed in the linear, quatrain likewise cubic enhancements of cis9, trans 11 (p<0.05) and trans 10, cis 12 CLA isomers (p<0.01). In comparison with the control, cis9, trans 11 CLA centesimal compositions of the probiotics treatment groups increased by 27.7, 40.4 and 23.4% for the 2.5, 5.0 and 7.5 g/h/day, levels, respectively (p<0.05).

In addition, trans 10, cis 12 CLA was not detected in the control, when they stepped up simultaneously to 0.07, 0.08 and 0.06 % for levels of 2.5, 5.0 and 7.5 g/h/day, respectively (p<0.01). About the C18:0, it was increased by tendency (p>0.05) in simultaneity with the clear reduction tendency of C18:2n6c (p>0.05) and significant subtraction of C18:3n3 by reason of additional probiotics (p<0.05).

Concerning with the very long-chain fatty acids (chain length greater than C18), with the exception of C24:1 kept unaffected and C22:6n3 run low by tendency (p>0.05), all the centesimal composition of other fatty acids was uplifted with linear, quadratic and also cubic

significance (C20:2: p<0.01; C20:3n3: p<0.05; C20:3n6: p<0.01; C20:4n6: p<0.05; C20:5n3: p<0.01; C24:0: p<0.01) (Table 9).

About the whole profiles of fatty acids in the plasma, Table 9 shown that the additional probiotics resulted in an increased tendency for Total Saturated Fatty Acid (TSFA) (p>0.05). An evident magnification for poly-Unsaturated Fatty Acid (pl-USFA) (p>0.05) and an overt incensement for desirable fatty acid contrasted with a trivial increment of mono-Unsaturated Fatty Acid (mo-USFA) (p>0.05) were observed. The supplementation of probiotics was also the reason for a faint enhancement of Total n6 fatty acid (Tn6) (p>0.05); a small reduction for Total n3 fatty acid (Tn3) (p>0.05); a small reduction for the pl-USFA: TSFA ratio; but a significant increment for the n-6: n-3 ratio.

Table 10 showed that when calculating the centesimal composition of plasma fatty acids into fatty acid (μg) contained in 1 mL plasma, the effects of probiotics on the fatty acid contents were principally the same in comparison with the centesimal composition that shown in Table 9.

On the whole, amongst all of the plasma fatty acids that were detected in this experiment, the increment of total saturated fatty acids centesimal composition was observed resulting from addition of probiotics (48.59, 48.58 and 49.04% vs. 47.6%), but kept those of C15:0, C16:0 and C17:0 face-off. At the same time, the addition of probiotics was in force for reducing C18-C22 polyunsaturated fatty acids and heightened the CLA content of plasma as anticipation.

When calculating the centesimal composition of plasma fatty acids into fatty acid (µg) contained in 1 mL plasma, the average contents of total saturated fatty acids (428.6, 441.5, 458.6 and 436.3 μg mL⁻¹ plasma for control, 2.5, 5.0 and 7.5 g/h/day probiotics treatments, respectively) showed increasing tendency (p>0.05). Of the desirable fatty acids, the amounts were 637.3, 660.0, 717.6 and 645.4 $\mu g \, \text{mL}^{-1}$ plasma for control, 2.5, 5.0 and 7.5 g/h/day probiotics treatments respectively, they showed an increment with linear significance (p<0.05). On the ratios of PUFA: SFA and n6: n3 the average values were 0.62, 0.58, 0.59, 0.59 and 2.58, 3.20, 3.33, 3.12 for control, 2.5, 5.0 and 7.5 g/h/day probiotics treatments, respectively, the ratio of PUFA: SFA decreased by tendency (p>0.05), but that of n6: n3 significantly increased (p<0.05). About CLA contents (µg mL⁻¹ plasma) of the four group animals, they were 4.2, 5.4, 6.4, 5.1 (μg mL⁻¹ plasma) and undetected, 0.6, 0.7, 0.5 (μg mL⁻¹ plasma) for cis9, trans11 and trans10, cis12 CLA isomer, respectively, the values of cis9, trans11 CLA presented a significant increment (p<0.01) and those of trans10, cis12 CLA showed a growing in number with highly significance (p<0.05).

DISCUSSION

The presence of probiotics (S. cerevisia and L. acidophilus) constituted a healthier and more favorable ruminal setting for digestive and absorption processes. And it is this healthier and more favorable ruminal setting to be responsible for the significant increase of DMI, ADG and feed efficiency. Whitley and Jackson (1994) reported that in their experiment, the ADG of growing goats was 30 g day⁻¹ for the probiotics treatment group compared to 10 g day⁻¹ for the control. In another study, El-Ghani (2004) observed highly significant elevation (p<0.01) for feed intake in bucks. In present year, Tripathi et al. (2007) stated that during the digestibility period of their experiment, an increased tendency (p>0.05) of DMI was found due to addition of yeast probiotics. More recently, Han et al. (2008) demonstrated that significant increases in DMI and ADG of growing

goats resulted from additional probiotics that contained *S. cerevisia* and *L. acidophilus*. The effectiveness of additional probiotics on DMI, ADG and feed conversion in goats were in the same case as in cattle.

The increase of dietary digestibility for addition of probiotics was the response of increasing colonization of fugal on plant cell; of stimulating growth or/and activity of fibrolytic bacteria; of increasing the activities of xylanase and pectinase and of establishing more favorable ecological conditions for growth and activities of the anaerobic autochtonous microflora. Chaucheyras et al. (1995) stressed that the addition of yeast cells increased the colonization of Neocallimastix frontalis fugal on plant cell and thereby increased cellulose degradation. The effectiveness of some yeast strains to stimulate growth or/and activity of fibrolytic bacteria has been pointed out (Dawson et al., 1990; Harrison et al., 1988). In addition, Chaucheyras and Fonty (2001) had proved that the S. Cerevisiae I-1077 has effect on establishment of fibrolytic bacteria; on degradation of a lignocellulosic substrate; on the main polysaccharide depolymerase and glycoside hydrolase activities of particle-associated microorganisms and on the development of the rumen digestive function. Recently, Feng et al. (2008) indicated that adding yeast culture increased the activities of xylanase and pectinase. Similar to the present study, Kumagai et al. (2004) had observed that in the condition of both of oat hay and high concentrate feeding, the presence of yeast probiotics tended to increase the digestibility of CP, CF and organic cell wall. Han et al. (2008) had pointed out that DM (p<0.01), Organic Matter (OM) (p<0.05) and NDF (p<0.05) digestibility was increased significantly with probiotics, CP digestibility showed an obvious increasing tendency. Moreover, others (El-Waziry et al., 2000; Martins et al., 2000) have reported the similar improvements of dietary digestibility. Besides these researches, many of other studies also, agreed with the findings (Dawson et al., 1990; Fadel Elseed and Abusamra, 2007; Feng et al., 2008).

The findings on pH were in accordance with those from the former studies, Doreau and Jouany (1998) have suggested that the supplementation of *S. cerevisiae* did not change ruminal pH. Moreover, many findings have emphasized that the yeast probiotics did not affect goats rumen pH value with any significance (Han *et al.*, 2008; Jiang *et al.*, 2008; Fadel, 2007; Galp, 2006; Dawson *et al.*, 1990). On the other hand, supplementation of *L. acidophilus* has shown to decrease ruminal pH (Krehbiel *et al.*, 2003). However, almost all former results showed that addition of probiotics maintained pH in the range that is compatible with the optimal ruminal ecologic dominance.

Supplementation of S. cerevisiae alone in the diet of goats has either let the NH3-N concentration down (Koul et al., 1998; El-Waziry et al., 2000; Galp, 2006), or kept it unaffected (p>0.05) (Corona et al., 1999; Tripathi et al., 2007; Jiang et al., 2008). From the results of Galp (2006), we can get the averages of ruminal fluid NH₃-N and blood urea that calculated from 0, 3 and 6 h post-feeding were 354.0, 308.3 (mmol L^{-1}) and 45.50, 43.00 (mg dL⁻¹) for control and S. cerevisiae treatment group respectively, there were no significant differences. The results of the present study showed that significant raise in NH3-N was caused by addition of probiotics, these findings were consistent with those of Fadel et al. (2007), which reported that S. cerevisiae resulted in a numerical increase in ammonia-N concentration. What is more, the present study also found significant raise in PUN and it agreed with the results of Galp (2006), which reported that the means of serum urea were 0.53 (8.9), 0.570(9.5) and 0.57(9.4) (g L⁻¹ and mmol L⁻¹) for control, 5 and 10 g day⁻¹ S. cerevisiae treatments, respectively, a significant difference was observed. In point of probiotics effects on ruminal fluid NH3-N concentration, it can be concluded that this effectiveness is dependent on composition of diet rather than the added doses of probiotics.

These results were similar as the previous studies. Thereunto, Fadel et al. (2007) reported S. cerevisiae resulted in a numerical increase in total VFA concentration. El-Waziry et al. (2000) reported that VFA concentration increased with yeast supplementation. El-Ghani (2004) elucidated in detail that ruminal VFA was significantly heightened for bucks fed S. cerevisiae at 6 h. In addition, many other researches on addition of S. cerevisiae in goats or lambs had explained the coherence of the results (Jiang et al., 2008; Tripathi et al., 2007; Chaucheyras and Fonty, 2001; Enjalbert et al., 1999). The effectiveness of additional yeast probiotics on production of VFA being that it has beneficial effects on growth and H2-utilisation of acetogenic bacteria (Chaucheyras et al., 1995) and since the acetogenic bacteria which produces acetate from CO₂ and H₂, the total VFA and acetic centesimal proportion should appear to be increased. However, in another experiment that was carried out in lambs (Chaucheyras and Fonty, 2001), even though total VFA was significantly higher in the S. cerevisiae group during the 20-50 days period, no any significant effect was observed on the centesimal composition of the major VFA mixture (acetate, propionate and butyrate) except that of acetate tended to increase. Han et al. (2008) also detected a significant increase of total VFA in probiotics supplemental group and in the meantime, no significant effect was observed on the centesimal composition of the major VFA mixture as well as the ratio of C₂: C₃ Krehbiel et al. (2003) reported that the supplementation of L. acidophilus has shown to

increase in ruminal propionate concentrations. This finding was opposite to the present study.

The previous findings for effect of S. cerevisia on ruminal protozoa were complicated. Thereof, Corona et al. (1999) reported that S. cerevisia did not change ruminal protozoa. Recently, Enjalbert et al. (1999) observed that S. cerevisiae treatment decreased Diplodinium sp. protozoa significantly but did not affect total protozoal counts. Presently, Tripathi et al. (2007) described that ciliate protozoa population did not change due to yeast supplementation. On the contrary, Jouany et al. (1998) found increase of protozoal count by occasion of addition of S. cerevisiae. Krehbiel et al. (2003) stated that supplementation of L. acidophilus has been shown to increase ruminal protozoal numbers, to change viable bacterial counts. In the same case, Han et al. (2008) reported the significant increment of protozoal and bacterial counts for the reason of supplementation of blend of S. cerevisiae and L. acidophilus probiotics. The results of this study were similar to the findings from Krehbiel et al. (2003), Galp (2006), Han et al. (2008).

Former studies on probiotics were devoid of data for N-balance of goats. More recently, one research on goats showed that N-intake, N-voided in faeces and urine and N-balance did not change due to supplementation of yeast (Tripathi *et al.*, 2007). The results of this study for N-balance had conformity with that of Tripathi *et al.* (2007). The enlarged urinary N and total N excretion observed in this study were related to the significant increment of ruminal NH₃-N and Plasma Urea N (PUN) concentration.

Up to now, no other research detailed the effect of probiotics on plasma fatty acid profiles. A similar research in Maltese goat kids found that the lactobacilli treatment significantly lowered the levels of blood Non-Essential Fatty Acid (NEFA) (p<0.001) and for triglycerides (p<0.05), but did not mention the fatty acid profiles. The increasing total plasma saturated fatty acids (p>0.05) centesimal composition. reducing polyunsaturated fatty acids (p<0.05 or p<0.01) and raising desirable fatty acids (p<0.05) resulted from the more effective ruminal biohydrogenation on account of addition of probiotics. The more effective ruminal biohydrogenation resulted in accumulation of saturated fatty acids and subtraction of polyunsaturated fatty acids in the rumen. Consequently, more saturated fatty acids and less polyunsaturated fatty acids went into the blood. The heightening CLA (p<0.01) was caused by the supplemented probiotics (S. cerevisiae and L. acidophilus) that stimulated the growth and/or activity of ruminal bacteria; accordingly more enzymes accumulated and acted on the substrates of CLA (linolein acid and linoleni acid). As a result, CLA was produced faster and the increasing accumulation appeared in the rumen, subsequently more CLA went into the blood. On

the other hand, the *L. acidophilus* itself has been well documented to produce CLA from linolein acid and linoleni acid (Kishino *et al.*, 2002; Julia *et al.*, 2006).

Additional probiotics (S. cerevisia and L. acidophilus) increased g/kg W0.75 dry matter intake (p<0.05), ADG (g day⁻¹) (p<0.01) and feed conversion (lowered ratio of DMI: ADG) (p<0.05); increased digestibility of NDF (p<0.05), EE, ADF and CP (p>0.05) as well as that of DM and OM (p>0.05). In the mean time, addition of probiotics unaffected ruminal average pH, but raised the NH₂-N and also PUN (p<0.05), increased TVFA (p>0.05), but reduced propionic proportion (p<0.05) and butyric proportion (p>0.05) in concurrent with raise of acetic proportion and C2:C3 ratio (p>0.05). Depressed ruminal protozoal number (p>0.05) and heightened ruminal total viable bacterial number were entailed by additional probiotics. Enlarged urinary and total N excretions were observed due to supplementation of probiotics. Supplementation of probiotics increased total saturated fatty acids (p>0.05), contrasted with decrease of C15:0 (p<0.01), C16:0 (p>0.05) and C18-C22 polyunsaturated fatty acids (p<0.05 or p<0.01) centesimal composition in plasma. In addition, supplemented probiotics was in force for heightening CLA (p<0.01); for raising desirable fatty acids (p<0.05); for reducing ratio of PUFA: SFA (p>0.05) and for raising ratio of n6:n3 (p<0.05).

CONCLUSION

We can claim that supplementation of probiotics was effectual for improvement of stall-feeding growing goats productive performances. There upto the levels of 2.5 and 5.0 g/h/day were tested-proof to be appropriated for improvement of growing goat rumen metabolism, growth performance and plasma CLA concentration.

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