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Supplementation of *Saccharomyces Cerevisiae* or *Lactobacillus Acidophilus* in Goats Diets

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Abstract**—**This experiment was performed with the purpose of investigating effect of additional blend of probiotics *Saccharomyces cerevisiae* and *Lactobacillus acidophilus* on 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. In the mean time, ruminal average pH unaffected, but the NH_3-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 ($p>0.05$). 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 ($p<0.05$).

Keywords**—**Probiotic, conjugated linoleic acid, plasma fatty acid, goats

I. Introduction

Aprobiotics 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* [1]. 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 [2]. 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 [3, 4]. The present experiment was carried out to study the effect of additional *S. cerevisiae* and *L. acidophilus* probiotics on 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.

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II. Materials And Methods

A. 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 I). Before experiment, the animals were injected with Ivomic (Merial Ltd., Iselin, NJ) for anti-internal parasite, and housed in individual pens (0.9 x 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/d probiotics (*L. acidophilus* about 2.0×10^{12} cfu/g, and *S. cerevisiae* about $5.0 \times 1\ 0^{11}$ cfu/g). 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 under the condition of maintenance plus lower activity and 50 g/d 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.

B 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 and *S. cerevisia* about 5.0×10^{11} cfu/g. 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%).

C.Chemical analysis and calculation

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 x 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. [5].

D. 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&W 122~3232 column was applied for determination of VFA, whereas a 100 m x 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 to 175 °C which lasted for 27 min. Continually it increased at 4 °C /min to 215 °C and kept for 31 min. Nitrogen was adopted as carrier gas with a 60 ml/min flow rate and the oven temperature was 250 °C. FID and injection temperature were fixed at 280 °C, and a 1μL injection was done with a 10-μL injector.

E. 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.

III. Results

As shown in Table I, 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%, 0.21% of the corn silage dry matter respectively.

Supplementation of probiotics did not conduce to significant changes for the ruminal average pH, howbeit the 5.0 g/h/d group was observed a decreasing tendency comparing to the control (6.42 vs. 6.72) ($p>0.05$) (Table II). Differed from the case of pH, ammonia nitrogen (NH_3-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) at the level of 7.5 g/h/d. 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 C_2 : C_3, 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).

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: $p<0.05$).

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 11CLA 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/d, 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/d, 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 IX).

About the whole profiles of fatty acids in the plasma, Table 3 illustrated 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 mild subtraction 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 IV 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 3. 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 436.3μg/ ml plasma for control, 2.5, 5.0, and 7.5 g/h/d 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 μg/ ml plasma for control, 2.5, 5.0, and 7.5 g/h/d 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/d 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 plasma) of the four group animals, they were 4.2, 5.4, 6.4, 5.1 (μg/ ml plasma) and undetected, 0.6, 0.7, 0.5 (μg/ ml 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 ($p<0.05$).

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 C18-C22 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 [6, 7].

IV. Conclusion

In the mean time, addition of probiotics unaffected ruminal average pH, but raised the NH_3-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. 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 ($p<0.05$).

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

Table I
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	0.60
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

TABLE II
THE EFFECT OF PROBIOTICS ON THE AVERAGE PH, AMMONIA NITROGEN (NH_3-N, MG/DL), PLASMA NITROGEN(PUN, MG/DL), AND VFA (MM/L) OF GROWING GOATS FED WHOLE PLANT CORN SILAGE

	Probiotics (g/h/d)				
	0	2.5	5.0	7.5	SEM
pH	6.72	6.63	6.42	6.58	0.06
NH_3-N	10.43^{b}	12.51^{a}	12.32^{a}	12.14^{a}	0.27
PUN	11.01^{b}	16.31^{a}	16.48^{a}	15.88^{a}	0.34
TVFA	56.22	56.82	56.93	59.28	0.70
VFA proportion (% TVFA)					
Acetate	66.28^{b}	67.82^{b}	68.37^{b}	69.23^{a}	1.09
Propionate	21.51^{a}	19.12^{b}	19.47^{b}	19.68^{b}	0.65
Butyrate	6.83	5.98	6.12	6.23	0.40
C_2:C_3	3.79^{b}	4.49^{a}	4.42^{a}	4.42^{a}	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 III
PLASMA FATTY ACIDS CENTESIMAL PROFILES OF GROWING GOATS SUPPLEMENTED PROBIOTICS UNDER CONDITION OF FEEDING WHOLE PLANT CORN SILAGE

	Probiotics (g/h/d)				
FA (%TFA)	0	2.5	5	7.5	SEM
C8:0	0.72^{a}	0.68 ^{a}	0.50^{c}	0.59^{b}	0.05
C10:0	0.15^{b}	0.29^{a}	0.26 ^{a}	0.22 ^{a}	0.03
C12:0	0.38^{b}	0.36^{bc}	0.50^{a}	0.26^{c}	0.04
C14:0	3.31^{b}	3.89 ^{a}	3.34^{b}	3.89 ^{a}	0.15
C15:0	0.45^{a}	0.39^{a}	0.17^{b}	0.23^{b}	0.05
C16:0	17.77	16.75	17.59	16.20	0.70
C16:1	0.84	0.88	0.81	0.77	0.08
C17:0	2.92	2.82	2.86	3.10	0.20
C18:0	22.74	23.04	23.15	24.26	1.11
C18:1n9t	1.88	1.87	1.96	1.87	0.06
C18:1n9c	16.60	16.41	17.07	17.58	0.70
C18:2n6c	15.80	15.10	15.44	15.35	0.70
C18:3n3	1.04 ^{a}	0.96^{a}	0.86^{b}	0.75 ^{b}	0.05
C18:c9,t11	0.47^{b}	0.60^{a}	0.66^{a}	0.58^{a}	0.03
C18:t10,c12	0.00^{b}	0.07^{a}	0.08^{a}	0.06^{a}	0.01
C20:2	0.95^{a}	0.60^{bc}	0.70^{b}	0.52^{c}	0.02
C20:3n3	2.82^{a}	2.21^{b}	2.37 ^{b}	2.57^{b}	0.12
C20:3n6	0.30^{a}	0.21^{b}	0.19 ^{b}	0.24^{b}	0.02
C20:4n6	3.11^{c}	3.94^{a}	3.51^{bc}	3.64^{ab}	0.24
C20:5n3	0.41 ^{a}	0.35^{b}	0.35 ^{b}	0.30^{c}	0.01
C24:0	1.16^{a}	0.27^{b}	0.21^{b}	0.29^{b}	0.10
C24:1	2.45	2.54	2.37	2.45	0.04
C22:6n3	3.36	3.13	3.34	3.15	0.18
TSFA	47.60	48.59	48.58	49.04	1.52
TMUSFA	21.77	21.70	22.51	22.67	1.07
TPUSFA	28.26	27.10	27.73	27.14	1.00
DFA	70.76	72.94	73.43	73.77	2.37
PUSFA/TSFA	0.59	0.56	0.57	0.55	0.01
Tn6	19.68	21.05	21.04	19.87	0.90
Tn3	7.63	6.65	6.92	6.77	0.09
n−6/n−3	2.58^{b}	3.05^{a}	2.91^{a}	2.94 ^{a}	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.

TABLE IV
FATTY ACID AND CONJUGATED LINOLEIC ACID CONTENTS (µG/ML PLASMA) IN PLASMA OF GROWING GOATS SUPPLEMENTED PROBIOTICS UNDER CONDITION OF FEEDING WHOLE PLANT CORN SILAGE

	Supplemented probiotics (g/h/d)				
FA (µg/ml plasma)	0	2.5	5.0	7.5	SEM
C8:0	7.0^{a}	6.5^{b}	6.9^{a}	6.8^{ab}	0.31
C10:0	1.3^{c}	2.6^{a}	2.6^{a}	1.9^{b}	0.26
C12:0	3.4^{b}	3.3^{b}	3.8^{a}	3.3^{b}	0.06
C14:0	29.8^{b}	35.2^{a}	32.6^{ab}	34.0^{a}	0.64
C15:0	4.1^{a}	3.5^{b}	2.6^{d}	3.0^{c}	0.25
C16:0	160	151.6	151.9	141.7	7.63
C16:1	7.6^{a}	8.0^{a}	7.9^{a}	6.9^{b}	0.47
C17:0	26.3^{ab}	25.5^{b}	27.9^{ab}	28.9^{a}	0.39
C18:0	196.8	208.5	226.2	212.2	11.0 8
C18:1n9t	16.9	16.9	17.1	16.4	1.03
C18:1n9c	149.5	148.5	146.8	153.8	3.14
C18:2n6c	152.3	145.7	150.6	143	3.01
C18:3n3	9.3^{a}	8.7^{a}	8.4^{ab}	6.5^{b}	0.66
C18:c9,t11	4.2^{c}	5.4^{b}	6.4^{a}	5.1^{b}	0.43
C18:t10,c12	0.0^{c}	0.6^{ab}	0.7^{a}	0.5^{b}	0.40
C20:2	8.6^{a}	5.4^{c}	6.9^{b}	5.8^{bc}	1.08
C20:3n3	25.4^{a}	20.0^{c}	23.2^{ab}	22.5^{bc}	0.79
C20:3n6	2.7	2.6	2.3	2.1	0.42
C20:4n6	28.1^{b}	35.6^{a}	34.3^{a}	33.1^{a}	0.91
C20:5n3	3.7^{a}	2.3^{c}	2.9^{b}	2.4^{c}	0.20
C24:0	10.4^{a}	4.3^{b}	4.1^{b}	4.5^{b}	1.07
C24:1	22.1	23.0	23.2	21.4	0.90
C22:6n3	30.2^{a}	28.4^{ab}	26.9^{b}	27.6^{b}	1.33
TSFA	428.6	441.5	458.6	436.3	5.15
TMUSFA	196.1	196.4	215.0	198.5	2.07
TPUSFA	264.5	254.9	272.6	258.6	1.02 10.7
DFA	637.3	660.0	717.6	645.4	9
PUFA/SFA	0.62	0.58	0.59	0.59	0.01
Tn6	177.3^{b}	190.1^{a}	204.3^{a}	183.8^{b}	5.03
Tn3	68.6^{a}	59.4^{b}	61.4^{b}	59.0^{b}	0.99
n−6/n−3	2.58^{b}	3.20^{a}	3.33^{a}	3.12 ^{a}	0.07

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.

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