

Effect of a whey protein and rapeseed oil gel feed supplement on milk fatty acid composition of Holstein cows

Article

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1 **Table 1.** Ingredients and chemical composition of experimental diets in both experiments (g/kg DM or as stated)

	Experiment 1	Experiment 2			
	TMR ¹	Control	WPG300 ¹	WPG600 ¹	WPG900 ¹
Ingredients					
Maize silage	267	375	375	375	375
Grass silage	209	125	125	125	125
Hay	36.0	-	-	-	-
Straw	18.0	15.0	15.0	15.0	15.0
Cracked wheat	-	128	110	92.4	74.8
DDGS wheat ²	118	42.6	42.6	42.6	42.6
Maize meal	76.0	-	-	-	-
Soyabean meal	48.0	57.1	59.7	62.2	64.8
Rapeseed meal	64.0	57.1	59.7	62.2	64.8
Palm kernel meal	41.0	32.0	32.0	32.0	32.0
Barley	44.0	-	-	-	-
Wheat feed	22.5	-	-	-	-
Maize distillers grains	22.7	-	-	-	-
Molassed sugar beet feed	-	32.3	32.3	32.3	32.3
Soybean hulls	-	84.2	84.2	84.2	84.2
Molasses	7.8	17.4	17.4	17.4	17.4
Bicarbonate	4.0	4.0	4.0	4.0	4.0

	Experiment 1	Experiment 2			
	TMR ¹	Control	WPG300 ¹	WPG600 ¹	WPG900 ¹
Salt	4.0	4.0	4.0	4.0	4.0
Limestone	4.5	2.0	2.0	2.0	2.0
Minerals	10.2	9.3	9.3	9.3	9.3
Whey protein	-	15.5	10.3	5.2	0.0
Whey protein gel	-	0.0	17.6 ³	35.2 ³	52.8 ³
Chemical composition					
DM (g/kg fresh)	-	577	568	558	550
Organic matter	-	898	899	901	901
Crude protein	-	173	173	176	179
Neutral detergent fibre	-	348	350	360	333
Acid detergent fibre	-	218	229	246	219
Starch	-	200	176	171	170
Water soluble carbohydrates	-	14.1	15.1	29.3	29.8
Ether extract	-	40.0	51.9	63.0	75.5
ME (MJ/kg DM)	-	11.9	12.3	12.7	12.9
Fatty acids					
16:0	-	3.7	3.9	4.1	4.2
18:0	-	0.55	0.65	0.76	0.80
<i>cis</i> -9 18:1	-	3.8	7.1	10.2	13.6

	Experiment 1		Experiment 2		
	TMR ¹	Control	WPG300 ¹	WPG600 ¹	WPG900 ¹
18:2 n-6	-	10.0	10.9	11.8	12.2
18:3 n-3	-	2.2	2.8	3.3	3.7
Total fatty acids	-	26.3	31.2	36.0	41.7

2 ¹ Where TMR excludes the added whey protein gel, rapeseed oil or whey protein isolate, and WPG300, WPG600 and WPG900 are treatment
3 diets containing whey protein gel of rapeseed oil included at 300, 600 or 900 g additional oil per cow per day. Control diet contains no
4 supplemental fat.

5 ² DDGS – Dried distillers grains and solubles.

6 ³ Oil content of 680 g/kg DM, comprising 61% cis-9 18:1, 18% 18:2 n-6, 9% 18:3 n-3, 4% 16:0 and 1.5% 18:0. Oil was low in glucosinolate.

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15 **Table 2.** Effect of supplementing dairy cow diets with either rapeseed oil or whey protein gel of rapeseed oil, on DM intake, milk yield and milk
 16 composition during Experiment 1 (units as specified)

	Least square means at day 8				Δ^1 (day 8 – day 0)			
	RO ²	WPG ²	SEM ³	<i>P</i> -value ⁴	RO ²	WPG ²	SEM ³	<i>P</i> -value ⁴
DM intake (kg/d)	23.7	23.0	1.35	0.256	1.2	1.3	0.088	0.938
Yield								
Milk (kg/d)	38.7	39.0	1.84	0.792	-0.75	0.90	0.817	0.319
Fat (g/d)	1423	1448	71.1	0.810	-39.5	44.0	83.82	0.664
Protein (g/d)	1129	1168	33.8	0.257	-21.3	47.0	10.89	0.073
Lactose (g/d)	1695	1768	84.9	0.307	-30.7	33.4	45.95	0.504
Casein (g/d)	819	848	28.9	0.280	-13.5	42.1	8.27	0.070
Concentration (g/kg)								
Fat	36.8	37.2	0.91	0.778	-0.43	0.47	1.689	0.803
Protein	29.6	29.8	1.44	0.076	0.14	0.47	0.404	0.593
Lactose	44.8	44.4	0.49	0.502	-0.007	-0.116	0.2573	0.834
Casein	21.5	21.8	1.21	0.002	0.16	0.56	0.300	0.225

	Least square means at day 8				Δ^1 (day 8 – day 0)			
	RO ²	WPG ²	SEM ³	<i>P</i> -value ⁴	RO ²	WPG ²	SEM ³	<i>P</i> -value ⁴
Urea (mg/kg)	307	289	27.2	0.050	-7.6	-18.0	17.26	0.603

17 ¹ Difference between measurements at day 8 and day 0 from each of two experimental periods.

18 ² Where RO and WPG were treatment diets formulated so that cows consumed 420 g/d of rapeseed oil as either oil (RO) or a whey protein gel of
19 rapeseed oil (WPG), respectively.

20 ³ SEM – standard error of the mean for n=8 measurements.

21 ⁴ Refers to the significance of overall effect of diet.

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30 **Table 3.** Effect of supplementing dairy cow diets with either rapeseed oil or whey protein gel of rapeseed oil, on milk fat concentration of the
 31 main milk fatty acids/groups during Experiment 1 (g/100 g fatty acids)

	Least square means at day 8				Δ^1 (day 8 – day 0)			
	RO ²	WPG ²	SEM ³	P-value ⁴	RO ²	WPG ²	SEM ³	P-value ⁴
4:0	2.8	2.8	0.06	0.865	0.05	0.07	0.05	0.750
6:0	1.8	1.7	0.07	0.439	0.07	0.11	0.045	0.638
8:0	1.1	1.0	0.06	0.598	0.05	0.11	0.033	0.482
10:0	2.4	2.3	0.22	0.627	0.02	0.20	0.080	0.300
12:0	3.0	3.0	0.28	0.659	-0.06	0.21	0.104	0.297
14:0	10.7	10.1	0.48	0.334	-0.17	0.08	0.442	0.805
16:0	28.4	26.3	0.88	0.036	-3.9	-2.9	0.65	0.474
<i>trans</i> -9 16:1	0.09	0.07	0.010	0.015	0.05	0.02	0.007	0.001
18:0	10.6	11.0	0.56	0.732	-0.86	2.96	0.913	0.164
<i>trans</i> -11 18:1	1.9	1.4	0.18	0.057	1.12	0.42	0.109	0.002
<i>cis</i> -9 18:1 ⁵	22.0	23.6	1.21	0.179	0.43	-0.81	0.553	0.743
<i>trans</i> 18:1 total	5.1	4.4	0.35	0.048	2.5	1.1	0.35	0.062

	Least square means at day 8				Δ^1 (day 8 – day 0)			
	RO ²	WPG ²	SEM ³	P-value ⁴	RO ²	WPG ²	SEM ³	P-value ⁴
<i>cis</i> 18:1 total	24.2	25.9	1.25	0.152	-0.11	-0.79	0.71	0.658
Non CLA 18:2 total ⁶	2.70	2.79	0.109	0.631	0.07	-0.12	0.154	0.568
CLA total	1.03	0.74	0.070	0.080	0.48	0.26	0.045	0.088
18:2 n-6	1.8	2.0	0.087	0.179	0.02	-0.13	0.129	0.516
18:3 n-3	0.40	0.43	0.009	0.161	-0.003	0.003	0.0075	0.494
Σ SFA ⁷	63.9	61.9	1.68	0.088	-4.20	0.03	0.779	0.086
Σ SFA \leq 14:0	22.0	21.3	1.06	0.418	-0.01	1.20	1.086	0.554
Σ <i>trans</i> total	7.0	5.7	0.56	0.140	3.1	2.0	0.25	0.031
Σ <i>trans</i> MUFA ⁸	5.8	5.0	0.42	0.002	2.7	1.2	0.42	0.084
Σ <i>cis</i> MUFA	26.5	28.0	1.29	0.180	0.05	-0.70	0.505	0.508
Σ n-6 PUFA ⁹	2.2	2.3	0.08	0.221	-0.06	0.04	0.064	0.126
Σ n-3 PUFA	0.77	0.73	0.022	0.119	0.11	0.04	0.046	0.340

32 ¹ Difference between measurements at day 8 and day 0 from each of two experimental periods.

33 ² Where RO and WPG were treatment diets formulated so that cows consumed 420 g/d of rapeseed oil as either oil (RO) or a whey protein gel of
34 rapeseed oil (WPG), respectively.

35 ³ SEM – standard error of the mean for n=8 measurements.

36 ⁴ Refers to the significance of overall effect of diet.

37 ⁵ Co-elutes with *trans*-13 and *trans*-14 18:1.

38 ⁶ CLA – conjugated linoleic acid. All 18:2 isomers excluding CLA.

39 ⁷ SFA – saturated fatty acids.

40 ⁸ MUFA – monounsaturated fatty acids.

41 ⁹ PUFA – polyunsaturated fatty acids.

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50 **Table 4.** Effect of incremental supplementation with whey protein gel of rapeseed oil on dry matter and fatty acid intake, and milk and
 51 constituent yield in Experiment 2 (least square mean results)

	Treatments ¹					<i>P</i> -value ²			
	Control	WPG300	WPG600	WPG900	SEM ³	Diet	LIN	QUAD	CUBIC
DM intake (kg/d)	24.0	23.6	24.6	23.5	1.43	0.025	0.632	0.077	0.008
Oil intake from WPG (g/d) ⁴	0	271	617	814	29.2	0.022	0.011	0.215	0.124
Fatty acid intake (g/d)									
16:0	89.1	91.7	99.8	100.5	5.73	0.001	0.001	0.431	0.038
18:0	13.3	15.4	18.5	19.0	1.00	0.001	0.001	0.014	0.012
<i>cis</i> -9 18:1	94.3	175.6	246.4	312.6	13.62	0.006	0.003	0.072	0.311
18:2 n-6	240	258	290	286	16.1	0.001	0.001	0.010	0.013
18:3 n-3	54.6	64.8	80.3	85.2	4.37	0.001	0.001	0.065	0.035
Total fatty acids	640	736	890	970	49.6	0.001	0.001	0.557	0.082
Yield									
Milk (kg/d)	36.8	37.6	40.7	40.8	2.15	0.009	0.002	0.634	0.118

	Treatments ¹					<i>P</i> -value ²			
	Control	WPG300	WPG600	WPG900	SEM ³	Diet	LIN	QUAD	CUBIC
Fat (g/d)	1424	1507	1504	1465	126.5	0.602	0.593	0.266	0.797
Protein (g/d)	1195	1213	1282	1260	79.7	0.171	0.064	0.483	0.270
Lactose (g/d)	1652	1708	1858	1863	126.8	0.045	0.010	0.608	0.220
Casein (g/d)	889	890	965	951	64.2	0.034	0.013	0.683	0.071
Concentration (g/kg)									
Fat	39.7	41.1	35.0	35.2	1.34	0.016	0.007	0.489	0.019
Protein	32.5	32.0	31.4	31.0	0.54	0.178	0.077	0.978	0.890
Lactose	44.8	45.6	45.5	45.4	1.17	0.225	0.182	0.113	0.609
Casein	24.3	23.7	23.8	22.8	0.38	0.111	0.060	0.268	0.168
Urea (mg/kg)	218	221	211	196	19.7	0.393	0.150	0.406	0.891

52 ¹ Where WPG300, WPG600 and WPG900 are diets containing 300, 600 or 900 g oil/d as a whey protein gel of rapeseed oil, respectively.

53 ² Refers to the significance of overall effect of diet or linear (LIN), quadratic (QUAD) and cubic (CUBIC) effects of increasing supplementation.

54 ³ SEM – standard error of the mean for n=16 measurements.

55 ⁴ Calculated using dry matter intake and oil content of the WPG supplement.

56 **Table 5.** Effect of incremental supplementation with whey protein gel of rapeseed oil on milk fatty acid composition in Experiment 2 (least
 57 square mean results as g/100 g fatty acids)

Fatty acid	Treatments ¹				SEM ³	P-value ²			
	Control	WPG300	WPG600	WPG900		Diet	LIN	QUAD	CUBIC
4:0	2.7	2.8	2.8	2.7	0.05	0.442	0.987	0.131	0.845
6:0	1.8	1.8	1.8	1.6	0.06	0.195	0.126	0.257	0.364
8:0	1.1	1.2	1.1	0.9	0.05	0.012	0.001	0.009	0.189
10:0	2.9	3.0	2.4	2.0	0.16	0.001	0.001	0.013	0.103
<i>cis</i> -9 10:1	0.34	0.26	0.23	0.21	0.026	0.080	0.028	0.294	0.632
12:0	4.0	3.7	3.4	2.9	0.22	0.03	0.001	0.295	0.626
<i>cis</i> -9 12:1	0.13	0.09	0.08	0.07	0.010	0.077	0.029	0.190	0.741
13:0	0.11	0.08	0.08	0.07	0.009	0.003	0.001	0.155	0.219
13:0 iso	0.03	0.03	0.03	0.02	0.001	0.416	0.122	0.722	0.905
13:0 anteiso	0.12	0.08	0.07	0.07	0.008	0.037	0.015	0.072	0.643
14:0	12.3	11.8	10.9	9.8	0.43	0.002	0.001	0.213	0.977
14:0 iso	0.06	0.06	0.06	0.06	0.004	0.754	0.329	0.915	0.790

Fatty acid	Treatments ¹					P-value ²			
	Control	WPG300	WPG600	WPG900	SEM ³	Diet	LIN	QUAD	CUBIC
<i>trans</i> -9 14:1	0.25	0.22	0.18	0.11	0.012	0.021	0.004	0.875	0.426
<i>cis</i> -9 14:1	1.21	1.15	0.94	0.89	0.077	0.086	0.044	0.892	0.170
15:0	1.14	0.87	0.82	0.72	0.076	0.028	0.009	0.267	0.390
15:0 anteiso	0.47	0.40	0.40	0.36	0.031	0.420	0.153	0.798	0.534
16:0	35.1	30.4	24.6	21.7	0.90	0.001	0.001	0.239	0.233
16:0 iso	0.18	0.18	0.16	0.16	0.015	0.413	0.142	0.902	0.405
<i>trans</i> -6+7+8 16:1	0.05	0.05	0.06	0.08	0.008	0.050	0.011	0.296	0.700
<i>trans</i> -9 16:1	0.04	0.05	0.07	0.08	0.006	0.002	0.001	0.797	0.312
<i>trans</i> -11+12+13 16:1	0.16	0.17	0.17	0.18	0.009	0.052	0.014	0.403	0.372
<i>cis</i> -9 16:1 ⁴	1.52	1.11	0.92	0.90	0.078	0.002	0.001	0.029	0.886
<i>cis</i> -11 16:1	0.50	0.43	0.42	0.38	0.024	0.020	0.005	0.399	0.339
<i>cis</i> -13 16:1	0.24	0.17	0.14	0.12	0.012	0.010	0.003	0.111	0.707
17:0	0.49	0.43	0.40	0.36	0.028	0.002	0.001	0.229	0.337
17:0 iso	0.34	0.31	0.31	0.29	0.019	0.059	0.013	0.572	0.445

Fatty acid	Treatments ¹					P-value ²			
	Control	WPG300	WPG600	WPG900	SEM ³	Diet	LIN	QUAD	CUBIC
<i>cis</i> -9 17:1	0.17	0.14	0.13	0.12	0.012	0.001	0.001	0.037	0.575
18:0	9.8	10.4	12.4	12.8	0.63	0.168	0.087	0.809	0.315
18:0 iso	0.05	0.03	0.03	0.03	0.012	0.490	0.326	0.290	0.541
<i>trans</i> 18:1 total	2.4	3.4	5.2	6.6	0.30	0.001	0.001	0.840	0.200
<i>cis</i> 18:1 total	16.9	20.1	24.3	26.6	0.67	0.001	0.001	0.444	0.256
Non CLA 18:2 total ⁵	2.4	2.7	3.0	3.4	0.12	0.009	0.002	0.602	0.792
CLA total	0.41	0.50	0.80	0.88	0.055	0.002	0.001	0.996	0.115
18:3 n-6	0.02	0.02	0.02	0.03	0.002	0.164	0.108	0.149	0.516
18:3 n-3	0.30	0.47	0.60	0.70	0.034	0.001	0.001	0.214	0.945
19:0 ⁶	0.08	0.07	0.10	0.11	0.013	0.088	0.035	0.247	0.255
20:0	0.14	0.18	0.21	0.22	0.009	0.001	0.001	0.067	0.903
<i>cis</i> -8 20:1	0.09	0.09	0.12	0.12	0.011	0.161	0.039	0.865	0.411
<i>cis</i> -11 20:1	0.04	0.09	0.14	0.18	0.012	0.006	0.001	0.927	0.681
20:2 n-6	0.04	0.04	0.04	0.05	0.002	0.291	0.081	0.712	0.402

Fatty acid	Treatments ¹					<i>P</i> -value ²			
	Control	WPG300	WPG600	WPG900	SEM ³	Diet	LIN	QUAD	CUBIC
20:3 n-6	0.09	0.08	0.08	0.07	0.008	0.002	0.001	0.422	0.540
20:3 n-3	0.01	0.03	0.04	0.05	0.008	0.022	0.005	0.775	0.850
20:4 n-6	0.12	0.11	0.10	0.09	0.010	0.012	0.002	0.683	0.970
20:5 n-3	0.04	0.04	0.03	0.03	0.001	0.063	0.016	0.292	0.667
22:0	0.05	0.05	0.06	0.06	0.004	0.614	0.222	0.854	0.749
22:4 n-6	0.02	0.02	0.02	0.02	0.003	0.997	0.982	0.828	0.898
22:5 n-3	0.09	0.08	0.09	0.06	0.021	0.840	0.483	0.798	0.786
Σ SFA ⁷	73.3	69.1	62.2	57.9	1.02	0.001	0.001	0.936	0.214
Σ SFA ≤14:0	25.4	24.3	22.8	20.2	0.88	0.001	0.001	0.043	0.584
Σ <i>trans</i> total	3.6	4.5	6.4	7.8	0.36	0.025	0.013	0.223	0.127
Σ <i>trans</i> MUFA ⁸	3.0	3.9	5.8	7.0	0.32	0.027	0.014	0.266	0.137
Σ <i>cis</i> MUFA	20.6	21.7	27.6	29.7	0.81	0.062	0.032	0.493	0.130
Σ n-6 PUFA ⁹	2.2	2.5	2.7	3.0	0.09	0.001	0.001	0.485	0.952
Σ n-3 PUFA	0.59	0.74	0.95	1.13	0.060	0.002	0.001	0.836	0.626

58 ¹ Where WPG300, WPG600 and WPG900 are diets containing 300, 600 or 900 g oil/d as a whey protein gel of rapeseed oil, respectively.

59 ² Refers to the significance of overall effect of diet or linear (LIN), quadratic (QUAD) and cubic (CUBIC) effects of increasing supplementation.

60 ³ SEM – standard error of the mean for n=16 measurements.

61 ⁴ Co-elutes with 17:0 anteiso.

62 ⁵ CLA – conjugated linoleic acid. All 18:2 isomers excluding CLA.

63 ⁶ Co-elutes with *cis*-15 18:1.

64 ⁷ SFA – saturated fatty acids.

65 ⁸ MUFA – monounsaturated fatty acids.

66 ⁹ PUFA – polyunsaturated fatty acids.

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73 **Table 6.** Effect of incremental supplementation with whey protein gel of rapeseed oil on milk fat 18:1 isomer composition in Experiment 2 (least
 74 square mean results as g/100 g fatty acids)

Fatty acid	Treatments ¹					P-value ²			
	Control	WPG300	WPG600	WPG900	SEM ³	Diet	LIN	QUAD	CUBIC
<i>trans</i> -4 18:1	0.00	0.02	0.05	0.06	0.005	0.001	0.001	0.319	0.363
<i>trans</i> -5 18:1	0.00	0.01	0.05	0.05	0.004	0.004	0.001	0.376	0.036
<i>trans</i> -6-8 18:1	0.25	0.38	0.72	1.00	0.046	0.001	0.001	0.039	0.086
<i>trans</i> -9 18:1	0.20	0.27	0.45	0.69	0.057	0.003	0.001	0.181	0.832
<i>trans</i> -10 18:1	0.40	0.83	0.95	1.93	0.234	0.107	0.057	0.209	0.228
<i>trans</i> -11 18:1	0.75	0.98	1.50	1.59	0.145	0.013	0.003	0.601	0.237
<i>trans</i> -12 18:1	0.38	0.45	0.55	1.14	0.154	0.067	0.023	0.186	0.537
<i>trans</i> -15 18:1	0.27	0.26	0.57	0.22	0.041	0.765	0.549	0.608	0.570
<i>trans</i> -16 18:1 ⁴	0.33	0.37	0.49	0.54	0.023	0.001	0.001	0.558	0.101
<i>cis</i> -9 18:1 ⁵	15.7	18.7	22.6	24.8	0.64	0.001	0.001	0.511	0.319
<i>cis</i> -11 18:1	0.59	0.67	0.81	0.89	0.068	0.001	0.001	0.832	0.130

Fatty acid	Treatments ¹					P-value ²			
	Control	WPG300	WPG600	WPG900	SEM ³	Diet	LIN	QUAD	CUBIC
<i>cis</i> -12 18:1	0.29	0.32	0.39	0.33	0.031	0.077	0.097	0.081	0.126
<i>cis</i> -13 18:1	0.09	0.10	0.12	0.14	0.008	0.001	0.001	0.190	0.260
<i>cis</i> -16 18:1	0.05	0.06	0.08	0.09	0.004	0.001	0.001	0.730	0.017

75 ¹ Where WPG300, WPG600 and WPG900 are diets containing 300, 600 or 900 g oil/d as a whey protein gel of rapeseed oil, respectively.

76 ² Refers to the significance of overall effect of diet or linear (LIN), quadratic (QUAD) and cubic (CUBIC) effects of increasing supplementation.

77 ³ SEM – standard error of the mean for n=16 measurements.

78 ⁴ Co-elutes with *cis*-14 18:1.

79 ⁵ Co-elutes with *trans*-13 18:1 and *trans*-14 18:1.

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85 **Table 7.** Effect of incremental supplementation with whey protein gel of rapeseed oil on milk fat non methylene-interrupted 18:2 isomer
 86 composition in Experiment 2 (least square mean results as mg/100 g fatty acids).

Fatty acid	Treatments ¹					P-value ²			
	Control	WPG300	WPG600	WPG900	SEM ³	Diet	LIN	QUAD	CUBIC
<i>cis</i> -9, <i>trans</i> -13 18:2	197	177	252	294	23.4	0.057	0.014	0.530	0.417
<i>cis</i> -10, <i>trans</i> -14 18:2	136.3	144.6	109.5	96.6	16.58	0.211	0.071	0.518	0.366
<i>cis</i> -9, <i>trans</i> -14 18:2	73.0	50.7	101.6	116.1	11.79	0.317	0.202	0.395	0.321
<i>cis</i> -9, <i>trans</i> -12 18:2	28.3	23.5	37.7	49.6	3.64	0.014	0.005	0.055	0.168
<i>trans</i> -9, <i>cis</i> -12 18:2	20.0	26.5	37.1	37.8	3.55	0.065	0.017	0.470	0.439
<i>trans</i> -11, <i>cis</i> -15 18:2	48.3	77.6	124.3	195.9	15.03	0.001	0.001	0.037	0.839
<i>cis</i> -9, <i>cis</i> -12 18:2	1817	2143	2385	2598	81.8	0.001	0.001	0.341	0.827

87 ¹ Where WPG300, WPG600 and WPG900 are diets containing 300, 600 or 900 g oil/d as a whey protein gel of rapeseed oil, respectively.

88 ² Refers to the significance of overall effect of diet or linear (LIN), quadratic (QUAD) and cubic (CUBIC) effects of increasing supplementation

89 ³ SEM for n=16 measurements

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