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Chicken extract affects colostrum protein compositions in lactating women

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Abstract

This study investigated the effect of supplementation with chicken extract on plasma and colostrum protein compositions in lactating women. Thirty healthy pregnant women were evenly divided into the control ($n = 15$) or chicken extract (CE) group ($n = 15$). The CE group was given one bottle (70 mL/bottle) of chicken extract three times a day to provide 18 g protein from the 37th week pregnancy to 3 days postpartum. All women in the CE group consumed chicken extract at least for 2 weeks (18 ± 5 days). High protein supplement was restricted in the control group. Blood samples were collected during the 37th week pregnancy and 3-day postpartum, and milk was collected during 3-day postpartum. The results showed that plasma total protein was significantly lower by 14% in the CE group compared with that in the control group during 3-day postpartum. Plasma epidermal growth factor (EGF) significantly elevated by 236% during 3-day postpartum compared with those during the 37th week pregnancy in the CE group. The levels of lactoferrin, EGF, and transforming growth factor-β2 (TGF-β2) in colostrum significantly increased by 34%, 62%, and 196%, respectively, in the CE group compared with those in the control group. However, the levels of total protein, casein, lactalbumin, and secretory immunoglobulin A in colostrum did not significantly differ between two groups. Therefore, supplementation with chicken extract increased colostrum levels of lactoferrin, EGF, and TGF-β2, which are important for the growth and immune functions of the infants, in lactating women.

Keywords: chicken extract, human colostrum, lactoferrin, epidermal growth factor, transforming growth factor-β2

1. Introduction

Human colostrum is rich in proteins, antibodies, growth factors, and maternal leukocytes to enhance fetal immune ability and resistance to infection [1]. Proteins in human milk include casein, lactalbumin, lactoferrin, immunoglobulin A (IgA), lysozymes, serum albumin, enzymes, and other minor proteins, which not only provide good sources of peptides, amino acids, and nitrogen for the infants but also play as antimicrobial or anti-inflammatory factors [2, 3]. Additionally, human milk contains certain growth-related factors, such as epidermal growth factor (EGF), insulin, insulin-like growth factor-I, -II, nerve growth factor, relaxin, transforming growth factor (TGF)-α, -β1, and -β2, and many of which appear to have a role in supporting infantile growth and development [3,4].

Chicken extract rich in proteins is traditionally consumed by the ill persons, pregnant and lactating women in Asian countries. Previous studies demonstrated that chicken extract enhanced iron absorption and utilization in rats [5] and humans [6]. Additionally, chicken extract has been reported to increase metabolic rate [7], lessen mental fatigue [8], activate neutrophils in humans [9], stimulate brain 5-hydroxytryptamine activity [10], and attenuate the chronic development of cardiac hypertrophy and arteriosclerosis in rats [11].

Few studies investigated the effect of chicken extract on lactation. A previous study found that the number of mothers with milk secretion on the first day postpartum was significantly higher in the chicken extract group who consumed two bottles (70 mL) of chicken extract for 3 days

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after parturition compared with that in the control or mixed Chinese medicine group who consumed 300 mL of lean pork soup with *Radix Astragali*, *Angelicae Sinensis Radix*, foshou, *Manitis Squama*, *Medulla Tetrapanacis*, and the fruit of *Vaccaria Segetalis Garcke* [12]. The number of mothers with milk production above the median value on the third day postpartum was also greater in the chicken extract group. The data suggest that chicken extract is effective in promoting early milk secretion and increasing milk production in lactating women. However, the levels of zinc, copper, iron, and calcium from maternal milk in the chicken extract group did not differ from those in the control and mixed Chinese medicine groups. It is not clearly known if supplementation with protein-rich chicken extract can increase certain milk proteins/peptides important for infantile growth and development in lactating women. Therefore, the purpose of this study was to further investigate the effects of chicken extract on the composition of milk and plasma proteins/peptides in lactating women.

2. Materials and methods

2.1. Subjects

The healthy pregnant women were screened by the physicians from the Department of Obstetrics and Gynecology at Taipei Medical University Hospital. The inclusive criteria for the subjects included: non-vegetarians, below 40 years old, gravidity less than 3, gestational age more than 37 weeks, natural delivery of single birth, free of any disease, no pregnant complication, and without a history of smoking, alcohol, and drug abuse during the pregnancy. After obtaining their consent forms, the pregnant women (23 to 39 years old) were divided into the control ($n = 15$) or chicken extract (CE) group ($n = 15$) according to their acceptability for chicken extract. The CE group was given one bottle (70 mL) of chicken extract, which was produced from steam-boiled whole chickens followed by centrifugation to remove fat and cholesterol, vacuum concentration to 3- to 4-fold, and sterilization by high temperature and pressure (prepared by Cerebos Taiwan Ltd., Taipei, Taiwan, R.O.C.) (Table 1), three times a day within 30 min after each meal from the 37th week pregnancy to 3 days postpartum. The daily amount of protein supplement from chicken extract was approximately 18 g. Chicken extract was given to the subjects in the CE group every week when they came back to the hospital for routinely pregnant examination. All women in the CE group consumed chicken extract at least for 2 weeks. During the experimental period, all subjects were allowed to maintain their regular diet, but meat concentrate and high protein supplement were restricted in the control group. The study was performed in accordance with the regulations of the ethics committee of Taipei Medical University Hospital.

Table 1
Compositions of chicken extract

Ingredient	Amount
	g/kg
Protein and peptide	83.0
Amino acid, free	3.1
Hexose	0.8
Fat	0.4
Mineral	mg/kg
Calcium	26
Iron	1
Magnesium	32
Zinc	2
Potassium	1740
Sodium	550
Chloride	1340
Phosphorus	480
Copper	2
Manganese	5
Sulfur	500
Selenium	0.05
Vitamin	mg/kg
Vitamin B_2	1.0
Vitamin B_6	0.37
Vitamin B_{12}	0.002
Niacin	6.4
Folic acid	0.15
Vitamin C	15

2.2. Sample collection and dietary intake

Non-fasting blood samples were drawn during the 37th week pregnancy and 3-day postpartum. Blood samples were collected in heparin-containing tubes, and centrifuged at 2,000*g* at 4°C for 10 min to separate cells and plasma. Milk was collected twice a day in the morning and afternoon by an electronic suction pump for 15-min suction of each breast from 1 to 3 days postpartum. The subjects were constrained breastfeeding 1 hr before milk collection, and the interval for milk collection was at least 4 hr. Pooled milk samples from each subject were skimmed by centrifuged at 4,000*g* at 4°C for 10 min.

To evaluate the effect on milk compositions caused by chicken extract supplement rather than by dietary intake, dietary intake was assessed in all subjects. The subjects were asked to record their 3-day dietary intake, including two weekdays and one weekend, twice at the 37th week pregnancy and during 3-day postpartum, respectively. Energy and nutrient intake was calculated by food composition software developed by Institute of Biomedical Sciences, Academia Sinica (Taipei, Taiwan, R.O.C.).

2.3. Biochemical measurements

Total protein in the plasma and milk was spectrophotometrically analyzed at 690 nm by the modified Lowry's method [13]. Plasma total cholesterol, LDL cholesterol, HDL cholesterol, and triacylglycerols were determined us-

ing a Hitachi Elecsys 7170S autoanalyzer (Tokyo, Japan) and standard enzymatic procedures (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Plasma iron and unsaturated iron binding capacity (UIBC) were spectrophotometrically determined by 2-nitroso-5-[N-n-propyl-N-(3-sulfopropyl)amino]phenol (nitroso-PSAP) reagent (Shinotest Co., Tokyo, Japan). Total iron binding capacity (TIBC) was calculated by adding the values of plasma iron and UIBC. Plasma ferritin was assayed by immunoturbidometry (Tinaquant®, Roche Diagnostics Co., Indianapolis, IN, U.S.A.). Plasma transferrin was assayed using a Beckman Array® 360 Protein System (Beckman Coulter, Inc., Brea, CA, U.S.A.). Plasma prolactin was measured by sandwich ELISA (Roche Diagnostics Co.) using a Hitachi Elecsys 2010 autoanalyzer.

Casein and lactalbumin levels in colostrum were determined by SDS-PAGE followed by Western blotting. Milk proteins (30 μg) were was mixed with an equal volume of 2× SDS-PAGE sample buffer (0.125 mol/L Tris-HCl (pH 6.8), 4% (w/v) SDS, 20% (v/v) glycerol, and 10% (v/v) 2-mercaptoethanol) [14], denatured at 100°C for 3 min, and applied to SDS-PAGE (Bio-Rad Mini-PROTEAN 3 Cell, Bio-Rad Laboratories, Hercules, CA, U.S.A.). Proteins were separated by 15% resolving gel with 4% stacking gel in the running buffer (25 mmol/L Tris (pH 8.3), 192 mmol/L glycine, and 0.1% (w/v) SDS) at 100 V for 1.5 hr. After separation on the gel, proteins were then transferred onto the nitrocellulose membrane (0.45 μm) using a semi-dry transfer unit (Hoefer TE 70, Amersham Biosciences Ltd. Taiwan Branch, Taipei, Taiwan, R.O.C.) in Towbin buffer (25 mmol/L Tris, 192 mmol/L glycine, 1.3 mmol/L SDS, and 10% (v/v) methanol) [15] at 200 mA for 1 hr. The membrane was washed briefly with phosphate buffered saline (PBS), and incubated with blocking buffer (50 g/L skim milk and 0.1% (v/v) Tween-20 in PBS) overnight. After blocking, the membrane was incubated with 0.5 mg/L mouse anti-human monoclonal casein antibody (Lab Vision Cor., Fremont, CA, U.S.A.) or sheep anti-human polyclonal lactalbumin antibody (Biogenesis Inc., Kingston, NH, U.S.A.) at room temperature for 1 hr. The membrane was washed three times with wash buffer (0.1% (v/v) Tween-20 in PBS), and incubated with 0.2 mg/L goat anti-mouse IgG-horseradish peroxide conjugate (Pierce Chemical Co., Rockford, IL, U.S.A.) for 1 hr. The blot was washed again three times with wash buffer, incubated with enhanced chemiluminescence (ECL™) Western blotting detection reagents (Amersham Biosciences Ltd. Taiwan Branch) for 1 min, and exposed to a x-ray film for 30 s. The bands were quantitated by an image analysis system (Gel analysis system, EverGene Biotechnology, Taipei, Taiwan, R.O.C.) and Phoretix 1D Lite software (Phoretix International Ltd., Newcastle upon Tyne, United Kingdom).

Lactoferrin level in milk was analyzed by a commercial ELISA kit (Bioxytech lactof enzyme immunoassay kit, Oxis International, Inc., Portland, OR, U.S.A.), and measured at 420 nm [16] using an ELISA reader (Multiskan RC, Labsystems Inc., Helsinki, Finland). Plasma and milk EGF and milk TGF-β2 levels were determined by commercial ELISA kits (Quantikine™ human EGF immunoassay kit #DEG00, DuoSet® human TGF-β2 ELISA development system kit #DY302, Research and Diagnostics Systems, Inc., Minneapolis, MN, U.S.A.), and detected at 450 nm and corrected at 540 nm [17,18]. Secretory IgA contents in the plasma and milk were analyzed by ELISA. Mouse anti-human secretory IgA (Sigma-Aldrich Co., St. Louis, MO, U.S.A.) was coated in a 96-well plate [19]. Secretory IgA level was assessed at 450 nm and corrected at 540 nm.

Table 2
Demographic and clinical characteristics of the mothers and infants[1]

	Control	Chicken extract
Maternal age[2], years	31.0 ± 5.4	31.0 ± 3.8
Gestational weight gain[2], kg	16.4 ± 5.2	15.8 ± 4.9
Gestational age[2], weeks	38.9 ± 1.0	38.9 ± 0.9
Parity[2], n	1.4 ± 0.5	1.1 ± 0.4
Primiparous[3], n	9 (60.0%)	13 (86.7%)
Infant sex[3]	9M, 6F	6M, 9F
Infant birth length[2], cm	51.3 ± 2.5	54.4 ± 2.3
Infant birth weight[2], g	3438 ± 205	3250 ± 397
Infant birth head circumference[2], cm	33.7 ± 0.9	33.4 ± 1.1

[1] Data are mean ± SD or number (n = 15). Data in the parentheses are the percentage of primiparous number.
[2] Compared by Student's *t* test.
[3] Compared by χ^2 test.

2.4. Statistical analysis

The data expressed as mean ± SD were analyzed by Microsoft Excel (Office 2000, Microsoft Cor., Redmond, WA, U.S.A.). Student's *t* and chi-square (χ^2) tests were used to analyze the continuous and discontinuous data, respectively, between the control and CE groups. Paired Student's *t* test was used to analyze the differences between the 37th week pregnancy and 3-day postpartum within the same group. Significant differences were considered when $P < 0.05$.

3. Results

3.1. Clinical data and dietary intake

No significant differences in the demographic and clinical characteristics of the mothers and infants were observed between two groups (Table 2). Daily dietary intake of the subjects during the 37th week pregnancy and 3-day postpartum is shown in Table 3. The duration for supplementation with chicken extract in the CE group was 18 ± 5 days (data not shown). During the 37th week pregnancy, dietary intake for fat, saturated and unsaturated fatty acids was significantly higher ($P < 0.05$) in the CE group excluding the supplement compared with the control group. The per-

Table 3
Daily dietary intake of the subjects during the 37th week pregnancy and 3-day postpartum[1]

	Control		Chicken extract			
			Exclude	Include	Exclude	Include
	Pregnancy	Postpartum	Pregnancy		Postpartum	
Energy						
kcal/d	1986 ± 293‡	2081 ± 373	2097 ± 449	2168 ± 449	1923 ± 206#	1994 ± 206
kJ/d	8301 ± 1225‡	8435 ± 1559	8766 ± 1877	9062 ± 1877	8038 ± 861#	8335 ± 861
Carbohydrate						
g/d	257 ± 63	257 ± 78	246 ± 77	246 ± 77	228 ± 52	228 ± 52
% energy	51.5 ± 8.1‡	49.0 ± 9.9	46.4 ± 9.0*	44.8 ± 8.8	47.5 ± 9.9	45.8 ± 9.6
Protein						
g/d	82 ± 18‡	101 ± 18‡#	91 ± 20	109 ± 20†	107 ± 30#	125 ± 30†
% energy	16.7 ± 3.4‡	20.1 ± 5.7‡#	17.7 ± 4.3	20.6 ± 4.6†	22.4 ± 6.4#	25.2 ± 6.3
Fat						
g/d	71 ± 16‡	73 ± 25‡	85 ± 24*	685 ± 24	61 ± 17*#	61 ± 17
% energy	32.4 ± 6.8	31.3 ± 7.7	36.3 ± 7.0*	35.0 ± 6.8	28.3 ± 7.1#	27.3 ± 6.8
Saturated fatty acids (SFA), g/d	23.1 ± 7.0‡	22.5 ± 11.3‡	28.0 ± 10.4*	28.0 ± 10.4	16.4 ± 5.3*#	16.4 ± 5.3
Unsaturated fatty acids (UFA), g/d	45.8 ± 11.8‡	49.4 ± 14.9†	54.0 ± 16.8*	54.0 ± 16.8	42.0 ± 12.1*#	42.0 ± 12.1
UFA/SFA ratio	2.1 ± 0.7	2.5 ± 0.7	2.1 ± 0.6	2.1 ± 0.6	2.7 ± 0.7#	2.7 ± 0.7
Niacin, mg N.E./d	14.0 ± 4.9‡	21.0 ± 6.2‡#	16.7 ± 6.9	25.7 ± 6.9†	25.0 ± 11.1#	34.0 ± 11.1†
Calcium, mg/d	587 ± 306	380 ± 270#	700 ± 350	704 ± 350	289 ± 201#	293 ± 201
Iron, mg/d	11.6 ± 5.3	13.7 ± 5.5	14.0 ± 7.1	14.2 ± 7.1	17.3 ± 10.3	17.5 ± 10.3
Sodium, mg/d	2936 ± 3561	1387 ± 902#	3738 ± 7425	3918 ± 7425	1048 ± 629#	1229 ± 629
Water, mL/d	1021 ± 327‡	1081 ± 246	1100 ± 432	1291 ± 432	986 ± 207	1177 ± 207†

[1] Data are mean ± SD (n = 15).
* Different from the control group during the same period ($P < 0.05$).
† Different from the chicken extract (CE) group excluding the supplement during the same period ($P < 0.05$).
‡ Different from the CE group including the supplement during the same period ($P < 0.05$).
# Differnet from the 37th week pregnancy within the same group ($P < 0.05$).

centage of total energy for carbohydrate significantly lower ($P < 0.05$) accompanied by increased percentage of total energy for fat ($P < 0.05$) in the CE group excluding the supplement compared with the control group. Supplementation with chicken extract significantly increased protein and niacin intake ($P < 0.05$) in the CE group, and significantly elevated energy, protein, fat, saturated and unsaturated fatty acids, niacin, and water intake ($P < 0.05$) compared with the control group. Additionally, the CE group had decreased percentage of total energy for carbohydrate ($P < 0.05$) with increased protein ($P < 0.05$) compared with the control group. During 3-day postpartum, dietary intake for fat, saturated and unsaturated fatty acids was significantly lower ($P < 0.05$) in the CE group excluding the supplement compared with the control group. Supplementation with chicken extract significantly raised protein, niacin, and water intake ($P < 0.05$) in the CE group, and significantly increased protein and niacin intake ($P < 0.05$), as well as decreased fat, saturated and unsaturated fatty acids ($P < 0.05$) compared with the control group. Both the control and CE groups excluding the supplement significantly increased protein and niacin intake ($P < 0.05$), but decreased calcium and sodium intake ($P < 0.05$) during 3-day postpartum compared with the 37th week pregnancy. Additionally, the CE group excluding the supplement significantly reduced energy, fat, saturated and unsaturated fatty acids intake ($P < 0.05$), and raised the ratio of unsaturated fatty acids to saturated fatty acids ($P < 0.05$) during 3-day postpartum compared with the 37th week pregnancy. Dietary intake for carbohydrate and iron was not influenced by the supplement and period.

3.2. Plasma lipids, iron status, and proteins

The CE group significantly lowered plasma total protein by 14% ($P < 0.05$) compared with the control group during 3-day postpartum (Table 4). However, plasma lipids, iron status, prolactin, EGF, and secretory IgA did not significantly differ between two groups during the 37th week pregnancy and 3-day postpartum. Plasma ferritin significantly increased ($P < 0.05$) and TIBC significantly decreased ($P < 0.05$) in both groups during 3-day postpartum compared with the 37th week pregnancy. Both plasma HDL cholesterol and transferrin significantly reduced by 12% ($P < 0.05$) in the control group during 3-day postpartum compared with the 37th week pregnancy. Plasma total cholesterol and LDL cholesterol significantly decreased by 14% and 20% ($P < 0.05$), and plasma EGF significantly elevated by 236% ($P < 0.05$) in the CE group during 3-day postpartum compared with the 37th wk pregnancy.

Table 4
Plasma lipid profiles, iron status, and levels of total protein, prolactin, epidermal growth factor, and secretory immunoglobulin A in the subjects during the 37th week pregnancy and 3-day postpartum[1]

	Control		Chicken extract	
	Pregnancy	Postpartum	Pregnancy	Postpartum
Plasma lipids				
Total cholesterol, mmol/L	6.35 ± 1.40	5.81 ± 1.38	6.00 ± 0.83	5.16 ± 0.76†
LDL cholesterol, mmol/L	4.15 ± 1.41	3.50 ± 1.34	3.75 ± 0.84	3.00 ± 0.80†
HDL cholesterol, mmol/L	1.62 ± 0.25	1.42 ± 0.23†	1.54 ± 0.24	1.42 ± 0.24
Triacylglycerols, mmol/L	2.49 ± 0.65	2.61 ± 0.90	2.61 ± 0.86	2.13 ± 0.60
Plasma iron status				
Iron, μmol/L	11.61 ± 5.65	9.74 ± 5.37	16.1 ± 8.3	12.4 ± 5.4
Ferritin, pmol/L	38.8 ± 23.8	110.1 ± 72.7†	49.6 ± 33.0	115.8 ± 74.1†
Transferrin, g/L	4.84 ± 0.61	4.24 ± 0.64†	4.73 ± 0.86	4.02 ± 0.89
Total iron binding capacity, μmol/L	80.8 ± 9.1	70.1 ± 10.1†	82.0 ± 13.2	68.1 ± 14.4†
Plasma proteins				
Total protein, g/L	96.2 ± 11.5	104.4 ± 16.0	99.4 ± 10.8	90.1 ± 17.2*
Prolactin, μg/L	435.4 ± 107.4	530.8 ± 173.1	373.7 ± 139.8	483.7 ± 159.4
Epidermal growth factor, μg/L	31.5 ± 24.5	44.8 ± 26.4	21.4 ± 7.6	72.0 ± 50.6†
Secretory immunoglobulin A, mg/L	22.2 ± 10.6	24.8 ± 17.4	22.3 ± 8.1	21.0 ± 4.0

[1] Data are mean ± SD (n = 15).
* Different from the control group during the same period ($P < 0.05$).
† Different from the 37th week pregnancy within the same group ($P < 0.05$).

3.3. Milk proteins

The levels of total protein, secretory IgA (Table 5), casein, and lactalbumin (Fig. 1) did not significantly differ between two groups. However, the levels of lactoferrin, EGF, and TGF-β2 in the CE group significantly increased by 34%, 62%, and 196% ($P < 0.05$), respectively.

4. Discussion

The supplemental period was from the 37th week pregnancy to 3 days postpartum, which includes the rapid growing period for the fetus and the initiation period for colostrum secretion. It is reasonable to assume that supplementation with chicken extract rich in proteins during that period may increase the nutrient intake for infantile growth and improve the quality of maternal milk. Infantile birth length, weight, and head circumference did not significantly differ between two groups, suggesting that supplementation with chicken extract at least for 2 weeks neither adversely affect the growth nor cause the overweight of the infants. Similarly, a previous study reported that the mean birth weight of mature infants did not significantly differ between the protein supplement and the control groups [20]. However, the protein supplement group significantly lowered the birth weight of premature infants. Different from our study, a single type of higher protein supplement at the dose of 40 g casein/day was given to a poor black urban population for a longer period (supplementation before 30 weeks gestation) in the previous study.

Supplementation with chicken extract significantly increased protein and niacin intake within the CE group and as compared with the control group during the 37th week pregnancy and 3-day postpartum. Additionally, higher dietary fat intake including saturated and unsaturated fatty acids in the CE group before supplementation with chicken extract was changed to lower dietary fat intake after supplement during 3-day postpartum compared with the control group. It suggests that chicken extract with high protein and niacin but with low fat may contribute to these results. Dietary intake for energy, protein, and niacin was closely to or at least met the Dietary Reference Intakes of Taiwan for the third trimester of pregnancy and lactation [21]. Dietary intake for calcium (~600 to 700 mg) and iron (~12 to 14 mg) of the subjects during the 37th week pregnancy was higher than the average intake of 456 mg and 11.2 mg, respectively, among the females aged of 25 to 34 years in Nutrition and Health Survey in Taiwan (NAHSIT, 1993 to 1996) [22], but was less than the Adequate Intakes for calcium (1000 mg) and Recommended Dietary Allowance

Table 5
The concentrations of total protein, lactoferrin, epidermal growth factor, transforming factor-β2, and secretory immunoglobulin A in colostrum of the subjects[1]

	Control	Chicken extract
Total protein, g/L	64.9 ± 26.1	75.6 ± 47.5
Lactoferrin, g/L	7.3 ± 3.0	9.8 ± 3.5*
Epidermal growth factor, μg/L	146.8 ± 73.5	237.6 ± 144.6*
Transforming growth factor-β2, μg/L	7.8 ± 5.8	23.1 ± 17.4*
Secretory immunoglobulin A, g/L	4.1 ± 1.3	4.8 ± 1.5

[1] Data are mean ± SD (n = 15).
* Different from the control group ($P < 0.05$).

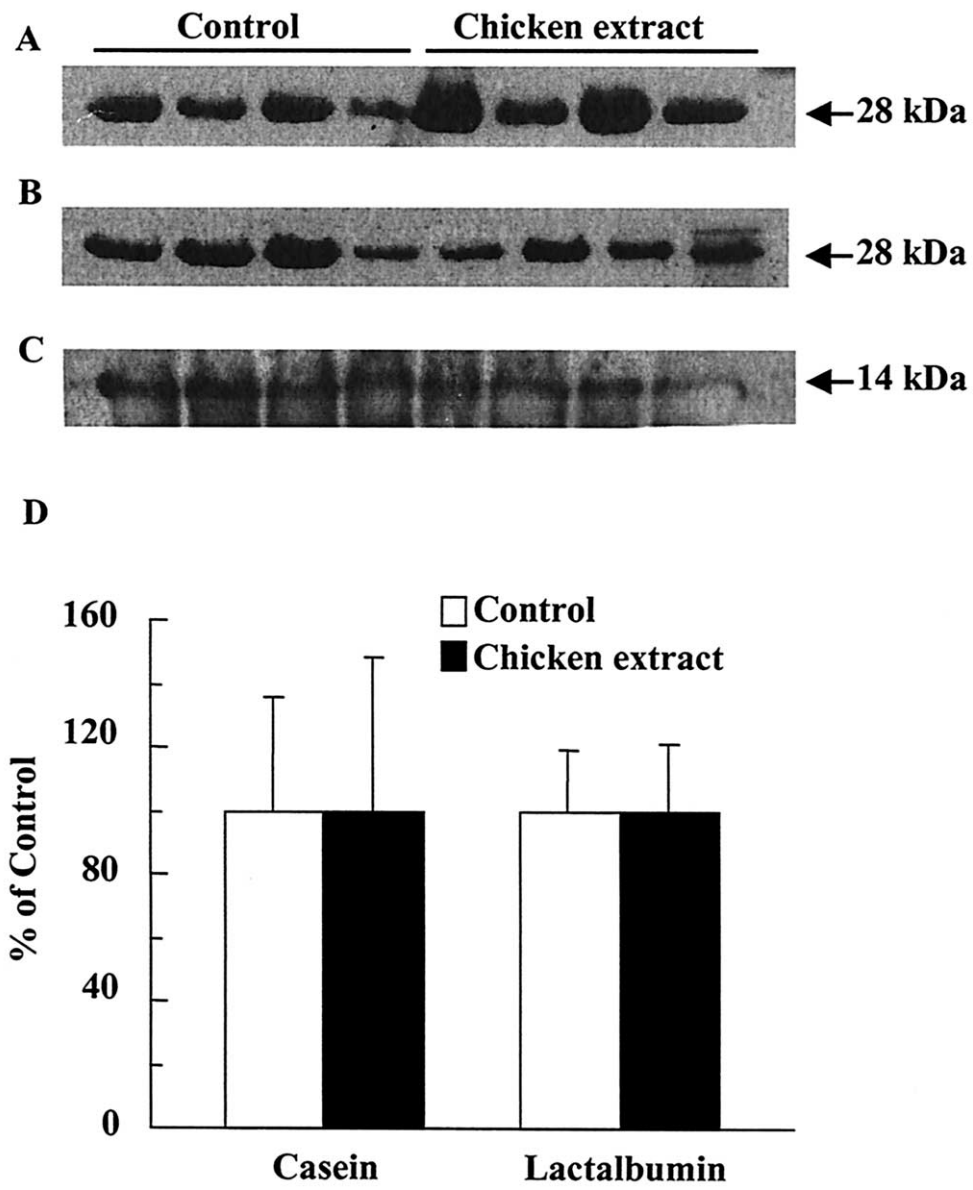

Fig. 1. Representative Western blotting for the expression of casein (A, B) and lactalbumin (C) with the molecular weight of 28 and 14 kDa, respectively, in colostrum of the control and chicken extract groups. The bands were then quantitated by an image analysis system (D). Values are the percentage of the control group (mean ± SD, $n = 15$). Both the expression of casein and lactalbumin in colostrum did not significantly differ ($P > 0.05$) between two groups by Student's t test.

for iron (45 mg). Inadequate calcium and iron intake was found in both the adult females and pregnant women in Taiwan. During 3-day postpartum, calcium intake even significantly fell in both groups compared with the 37th week pregnancy, probably because of too much forbidden food in traditional Chinese food aversion during postpartum.

The levels of plasma total cholesterol, LDL cholesterol, and triacylglycerols in the subjects during the 37th week pregnancy were higher than the favorable prognosis ranges (<5.17, <3.88, and <2.29 mmol/L for total cholesterol, LDL cholesterol, and triacylglycerols). Similar to our results, the levels of plasma total cholesterol, LDL cholesterol, and triacyglycerols significantly increased in the third trimester of pregnancy compared with those in the first trimester of pregnancy (7.4 ± 1.2 vs. 4.6 ± 0.8 mmol/L total cholesterol, 4.5 ± 0.9 vs. 2.6 ± 0.6 mmol/L LDL cholesterol, 2.2 ± 0.5 vs. 1.0 ± 0.3 mmol/L triacylglycerols, $P < 0.01$) [23]. A moderate increase in plasma total cholesterol, LDL cholesterol, and triacylglycerols, primarily because of the increases in 17β-estradiol and human placental lactogen, has been consistently described during pregnancy and postpartum [23, 24]. Supplementation with chicken extract significantly lowered total cholesterol and LDL cholesterol within the favorable prognosis ranges during 3-day postpartum, which was accompanied by reduced dietary fat intake. However, plasma HDL cholesterol sig-

nificantly decreased in the control group during 3-day postpartum.

Plasma iron status in both groups was near iron depletion during the 37th week pregnancy. Because of hemodilution and an increase in erythropoiesis, serum iron and ferritin levels decrease, and transferrin and TIBC levels increase during pregnancy [25, 26]. Although the subjects did not have iron deficiency anemia, iron supplementation may be recommended during pregnancy because of their low dietary iron intake. Both the control and CE groups had similar trends for the changes of plasma ferritin, transferrin, and TIBC levels during postpartum compared with the 37th week pregnancy, suggesting that it may be resulted from the physiological changes during postpartum rather than from the supplement. It has been reported that serum ferritin concentration increases quite rapidly in the postpartum period [27].

Plasma total protein and prolactin levels were greater in the subjects compared with the normal ranges (66 to 87 g/L for protein, $\leq$ 25 μg/L for prolactin). Plasma protein synthesis in the liver increases under the influence of estrogenic hormones, and the approximate 20% increase in the pool of circulating plasma protein, particularly albumin [28]. Although plasma total protein fell in the CE group during 3-day postpartum, the level of plasma total protein was still above the normal range. Because parity tended to be less in the CE group, it is suspected that plasma proteins decreased in the CE group during lactation directly proportional to parity [29]. However, a previous study demonstrated that plasma levels of total protein did not change in the third trimester of pregnancy between primigravid and multigravid women, but significantly increased in primigravid women who exhibited a bimodal frequency distribution during lactation (4 ± 2 days postpartum) [30]. The concentration of circulating prolactin, which plays major roles in maintaining the corpora lutea of pregnancy and in milk production and secretion during lactation, increases progressively throughout pregnancy to 10 to 20 times over normal value, and declines to non-pregnant levels by 3 to 4 weeks postpartum [31]. It supports that plasma prolactin increased to 15 to 21 times over normal value during the 37th week pregnancy and 3-day postpartum in our study. Supplementation with chicken extract significantly increased plasma EGF during 3-day postpartum compared with the 37th week pregnancy. It is suspected that increased plasma EGF contributes to elevated milk EGF which may be derived from the maternal circulation or from mammary synthesis [32], and chicken extract may stimulate the production of EGF in response to increased milk EGF.

Consistent with the results of plasma proteins, chicken extract significantly increased EGF level in colostrum, but had no effect on the concentration of secretory IgA. Additionally, chicken extract significantly elevated lactoferrin and TGF-β2 levels in colostrum. A previous study showed that lactogenic hormones prolactin and hydrocortisone increased EGF mRNA expression in mouse mammary glands [33]. However, plasma prolactin level did not change in the CE group, increased EGF in colostrum may not result from the stimulation of prolactin. The levels of lactoferrin, EGF, and TGF-β2 in colostrum could be influenced by each other. The expression of lactoferrin in mouse uterus was regulated by EGF [34], and TGF-β1 stimulated lactoferrin release from human neutrophils [35]. Whereas the mechanisms for increased lactoferrin, EGF, and TGF-β2 by chicken extract are not clearly known. It is suspected that histidine-containing dipeptides, anserine and carnosine abundantly occurring in animal organization, especially in chicken extract may play a role in the stimulation of these factors production. Anserine and carnosine have been found to possess strong antioxidant properties, act as neurotransmitters and immunomodulating agents, accelerate wound healing, regulate enzymatic activities, and chelate heavy metals [36,37].

Supplementation with chicken extract during the third trimester of pregnancy and early postpartum increases protein and niacin intake and decreases fat intake, and consequently lowers plasma total cholesterol and LDL cholesterol levels. Additionally, chicken extract elevates the concentrations of plasma EGF, colostrum lactoferrin, EGF, and TGF-β2 without affecting the levels of plasma prolactin, secretory IgA, colostrum total protein, casein, lactalbumin, and secretory IgA. Lactoferrin, an iron-binding glycoprotein in milk, has been proposed to play a role in iron uptake by the intestinal mucosa and to act as a bacteriostatic agent by withholding iron from iron-requiring bacteria [38]. Additionally, lactoferrin is involved in phagocytic killing and immune responses, and as a growth factor and as a bactericidal agent. Both EGF and TGF-β play roles in gastrointestinal (GI) development of the neonate, the immunological protection of the GI mucosal surface, and in prevention of tissue injury and acceleration of wound repair [32,39]. Overall, supplementation with chicken extract is beneficial not only to lactating women in lowering plasma lipids, but also to the breastfeeding infants, especially the premature or very-low-birth-weight infants, in facilitating the maturity of GI development, and in enhancing the defensive ability of the immune functions by improving milk quality.

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