Global Veterinaria 4 (5): 434-440, 2010
ISSN 1992-6197

Influence of Various Levels of Energy and Protein on Performance and Humoral Immune Responses in Broiler Chicks

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Abstract: Six hundred and forty day-old broiler chicks (Ross 308) were divided into 80 groups of 8 birds each. A 4×4 factorial arrangement in a complete randomized design experiment was used to study the effect of four levels of energy (2900, 3000, 3100 and 3200 kcal/kg) and four levels of protein (17, 20, 23 and 26%) on performance and humoral immune responses of chickens. Each diet was randomly fed to five groups of chicks from 5 to 35 d of age. Group weight and feed intake were recorded every 5-day. Lymphoid organs and liver weight were determined at 10, 15 and 20 d. Five birds were intramuscularly injected with 1 ml/chick sheep red blood cell (SRBC) 15% suspension in PBS at days 15 (primary injection) and 25 (secondary injection) of age. Blood samples were collected 5 and 10 days after each injection and subsequently were evaluated for total immunoglobulin, IgM and IgG anti-SRBC titers. Chicks BW and FCR improved as dietary energy and protein increased in every period and whole experimental period. The chicks feed intake was not influenced by dietary protein level. Any considerable effects of dietary energy and energy levels on lymphoid organ weights were observed. Broiler fed low levels of protein had heavier liver weight than those fed diets with high levels. Total and IgG anti-SRBC antibody titers were rose in birds fed low energy diets at postprimary injection (PPI) days. Immunoglobulin M titers were not significantly altered by different levels of energy at both PPI and PSI days. Dietary protein contents did not influence bird's anti-SRBC antibody titers. Protein and energy of diet are equally important for early growth and feed efficiency. Whereas, there are negative phenotypic contribution between immunocompetence and rapid growth in chickens, rapid growth decreased immune responses. This means that birds were selected for rapid growth but not for enhanced immune responses.

Key words: Energy · Protein · Humoral immune response · Broiler chicks

INTRODUCTION

Over the past 40 to 50 years, the poultry industry has progressed because of the improvements in genetic [1, 2], nutrition [3] and control of environmental factors. Genetic selection for high production potential in poultry has had some negative consequences [4]. A negative consequence of high growth rate in modern broiler strains is increase of susceptibility to disease [5, 6]. For instance, body weight was negatively correlated to antibody response to SRBC [6,7] and resistance to marek's disease [8]. Cheema *et al.* [9] found that the humoral immune responses of a current commercial broiler had declined than Athens-Canadian randombred control strain (developed from early broiler strains in 1957) over the years, whereas cellular functions including macrophages and natural killer cells were not affected.

Interactions between nutrition and immunity are diverse and have profound implications on animal growth and productivity. Many researchers demonstrated the effects of energy, protein [10], Zinc [11], copper [12], Selenium [13], vitamin A [14], E [15] and D [16] on immune responses of broiler chicks. Takahashi *et al.* [17] observed greater acute-phase immune response, as measured by plasma α1-acid glycoprotein and interleukin-1-like activity, in response to *Escherichia coli* lipopolysaccharide (LPS) in chicks raised on a low-protein diet in comparison with those fed a high-protein diet. Rao *et al.* [18] reported genotypes with heavier body weights had significantly higher lesion scores and lower antibody titers than those with less body weight. Also, genotypes of lower body weight had a greater cutaneous basophilic hypersensitivity response to phytohaemaglutinin-P inoculation and a better humoral

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response against SRBC and a lower heterophil to lymphocyte ratio. On the whole, energy and protein are known as two so effective nutrients in broiler growth rate also have indirect influence on immune response by hormone interfere. The purpose of this study was to investigate the effect of different levels of energy and protein on performance, lymphoid organ weights and humoral immune responses in broiler chicks during rapid growth.

MATERIALS AND METHODS

A complete randomized design experiment with a 4×4 factorial arrangement consisted of four levels of energy (2900, 3000, 3100 and 3200 kcal/kg) and four levels of protein (17,20, 23 and 26%) was conducted to assess the effects of dietary energy and protein on performance and humoral immune responses of broiler chickens. Broiler chicks were fed a commercial broiler starter diet from 1 to 4 d of age. Six hundred forty chicks were selected by weight from a 2000 population to minimize variability in cage weight. Chicks were assigned to 80 battery unit of 8 birds each on day 5. Each of the sixteen experimental diets was randomly allotted to 5 battery units from 5 to 35 d of age. The main ingredients of experimental diets were corn, soybean meal, wheat bran, corn gluten meal and soybean oil (Table 1). An inert filler (washed sand) was used to maintain specified nutrient ratios across all CP and ME levels. House temperature was decreased by 2.5°C per week starting approximately with 32°C at the day of arrival (d-old chicks) to a final temperature of 22°C at 28 d of age and there was no control on relative humidity. Broilers were reared under continuous incandescent lighting. Birds had access to jug and nipple waterers for the first week, after which the jug waterers were removed. Feed (in mash form) and water were provided ad libitum.

Body weight, mortality and feed intake per unit were recorded each 5-day intervals and the feed conversion ratio (FCR) was calculated. One chick per unit was slaughtered on d 10, 15, 20 and Spleen, bursa of Fabricius and liver were removed, weighed and expressed as a percentage of BW. All birds in each replicate unit were intramuscularly injected with one ml/chick sheep red blood cell (SRBC) 15% suspension in PBS at d 15 (primary injection) and 25 (secondary injection). Blood samples were collected from brachial vein 5 and 10 days after each injection. The serum from each sample was collected; heat inactivated at 56°C for 30 min and then analyzed for total, IgG (mercaptoethanol-resistant) and IgM (mercaptoethanol- sensitive) anti-SRBC antibodies as described by Cheema *et al.* [9].

Table 1: Composition (g/kg) of experimental diets

	2900				3000				3100				3200			
Ingredients	17	20	23	26	17	20	23	26	17	20	23	26	17	20	23	26
Corn	66	63.2	55.49	48	64.15	62.72	56.25	52.9	65.1	60.65	56.65	50.57	63.54	58.45	54	50.2
Corn gluten meal	0	1.9	9.1	16	0	2.4	10	16.6	0	2.65	9.7	16.7	0	2.95	10.64	17.2
Wheat bran	8.2	3.7	6.6	8.54	8	2.4	4.45	3.15	5	2.4	2	3.4	4.3	2.4	2	1.96
Soybean meal	19.5	26.03	23.8	22.4	19.8	25.9	23.3	22.5	20.8	25.9	24.4	22.6	21.4	26	24.34	22.6
Vegetable Oil	0.7	0	0	0	2.48	1.37	1	0	3.52	3.18	2.24	1.86	5.19	5	4	3.2
Limestone	1.74	1.7	1.78	1.8	1.73	1.7	1.72	1.71	1.68	1.66	1.68	1.73	1.68	1.66	1.67	1.7
Di Calcium phosphat	1.25	1.27	1.15	1.08	1.25	1.29	1.22	1.22	1.34	1.33	1.28	1.21	1.34	1.32	1.3	1.24
Nacl	0.3	0.28	0.28	0.27	0.29	0.29	0.27	0.27	0.3	0.3	0.27	0.28	0.3	0.3	0.28	0.27
Methionin 98	0.46	0.33	0.2	0.06	0.47	0.33	0.19	0.04	0.45	0.33	0.18	0.05	0.45	0.32	0.17	0.03
Hcl Lysin	0.47	0.25	0.26	0.24	0.49	0.26	0.28	0.26	0.47	0.26	0.26	0.26	0.46	0.26	0.26	0.26
Vitamin Mix[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral mix[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Filler (washed sand)	0.78	0.74	0.74	1	0.74	0.74	0.72	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74
vitamin E	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Calculated composition (%)																
AME (kcal/kg)	2900	2900	2900	2900	3000	3000	3000	3000	3100	3100	3100	3100	3200	3200	3200	3200
Crude protein	17	20	23	26	17	20	23	26	17	20	23	26	17	20	23	26
Calcium	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Available phosphor	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Lysine	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Methionin+Cystine	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97
Linoleic acid	1.82	1.54	2.08	2.58	2.67	2.24	2.63	2.64	3.16	3.12	3.2	3.54	3.96	4.02	4.1	4.2

[1]Supplied per kilogram of diet: vitamin A, 10,000 IU; vitamin D3. 9800 IU, vitamin E. 121 IU; B12, 20 ig; riboflavin, 4.4 mg; calcium pantothenate, 40 mg, niacin, 22 mg; cholin, 840 mg; biotin, 30 µg, thiamine, 4 mg; zinc sulphate, 60 mg; manganese, 60 mg

Statistical Analysis: The data were subjected to ANOVA as a completely randomized design using the GLM procedure of SAS software [19]. Energy, protein and two way interactions were included in all analyses. Anti-SRBC titers and lymphoid organ weights data were transformed to log2 and arc sin, respectively. Means were separated by Tukey's test at significance level of *P*<0.05.

RESULTS AND DISCUSSION

Performance: The effect of various levels of energy and protein on performance parameters is shown in Table 2. There were no energy and protein significant interactions on performance parameters (P<0.05) in every/or whole experimental period, with the exception of feed intake and FCR during d 30 to 35 (P=0.004) and 20 to 25 (P=0.03), respectively. Broiler chicks BW and FCR were improved with increasing energy and protein level of diets during whole experimental period (P<0.05). The feed intake was increased in parallel to the increase in dietary energy, subsequently chicks had high levels of energy and protein diet had higher feed intake (P<0.05) than those fed low levels up to 25 d. Richards [20] and Latshaw [21] found the modern commercial broiler, which selected for rapid growth and enhanced muscle mass, does not adequately regulate voluntary feed intake to achieve energy balance. Decreasing dietary CP below a minimum level (in this experiment, 20%), retarded growth and increased FCR. The results of present study are in agreement with other reports [22-27], which demonstrated the negative effect of protein deficiency on broiler performance. On the other hand, diet formulated to have 26% protein did not have a significant effect on BW, FCR and feed intake as compared to those fed 23% protein diet. It seems that the extra nitrogen was excreted as uric acid and ammonia. Other investigators reported N excretion and ammonia production in birds fed high CP diets [26, 28-30].

Relative Organ Weights: The effect of various levels of energy and protein on relative organ weights is shown in Table 3. The Bursa of Fabricius and spleen relative weights were not significant different in chicks fed diets with various levels of energy and protein. There was not an interaction effect of energy by protein on spleen, bursa of Fabricius and liver at d 15 and 20, although a significant interaction was found on spleen (P<0.01) and bursa of Fabricius (P=0.025) relative weights on d 10. Broiler fed low levels of protein had heavier liver weight than those fed diets with high levels. Chicks fed diet with low CP content showed to decrease relative weight of bursa of Fabricius [18, 31], which it does not in cooperation to

Table 2: Effect of various levels of dietary energy and protein on broiler chicks performance parameters

		ME					CP					Source of variation		
		2900	3000	3100	3200	SEM	17	20	23	26	SEM	ME	CP	ME*CP
Feed Intake						(g/d/bird)							Probability	
	5-10 d	21	20.6	21.1	21.3	0.39	21.3	20.9	20.8	21	0.39	0.63	0.84	0.82
	10-15 d	52.2^{b}	53.4^{ab}	53.6^{ab}	56.7^{a}	1.11	53.3	54.7	53.7	54.1	1.11	0.04	0.84	0.78
	15-20 d	58.8^{b}	60.2^{ab}	62^{ab}	64.8^{a}	1.16	60.9	60.9	62.9	61.1	1.16	0.004	0.54	0.40
	20-25 d	72.3^{b}	76.3^{ab}	74.4^{ab}	81.6^{a}	2.27	73	77.3	78.8	75.6	2.27	0.03	0.3	0.66
	25-30 d	108	104	112	107	2.49	105.6	109.9	113.5	110	3.45	0.14	0.09	0.32
	30-35 d	134	136	135	138	4.39	141.2	132.7	135.3	134	4.39	0.96	0.54	0.004
	5-35 d	74.4^{b}	75.6^{ab}	76.5^{ab}	78.3^{a}	0.95	74.7	76	77.5	76	0.95	0.027	0.23	0.39
Body weight						(g)								
	5 d	100.6	100.3	100.8	100.2	0.2	100.6	100.3	100.8	101.6	0.238	0.25	0.2	0.18
	10 d	159.6	163.5	163.8	164.8	2.1	155^{b}	161.4^{ab}	167.3^{a}	168^{a}	2.1	0.32	<.001	0.9
	15 d	294.7^{b}	313^{ab}	311^{ab}	323.7^{a}	5.2	283.7^{c}	306.2^{b}	323.5^{ab}	328.9^{a}	5.2	0.003	<.001	0.58
	20 d	439.1^{c}	474.8^{b}	490.1^{ab}	508.9^{a}	7.91	429.5^{c}	471^{b}	500.7^{a}	510.6^{a}	7.9	<.001	<.001	0.79
	25 d	619.5^{c}	677.1^{b}	708.3^{ab}	749.3^{a}	11.83	613.8^{c}	685.5^{b}	721.2^{ab}	733.6^{a}	11.3	<.001	<.001	0.25
	30 d	883.5^{c}	955.4^{b}	1011.8^{ab}	1043.5^{a}	17.31	860.6^{b}	970.7^{a}	1031^{a}	1031^{a}	17.3	<.001	<.001	0.45
	35 d	1209^{c}	1289^{b}	1357.7^{ab}	1410^{a}	21	1191^{c}	1304^{b}	1392^{a}	1379^{a}	21	<.001	<.001	0.79
FCR						(g:g)								
	5-10 d	1.83^{a}	1.69^{ab}	1.73^{ab}	1.66^{b}	0.041	1.97^{a}	1.73^{b}	1.57^{b}	1.62^{b}	0.041	0.025	<.001	0.92
	10-15 d	1.94^{a}	1.83^{ab}	1.85^{ab}	1.8^{b}	0.039	2.08^{a}	1.91^{b}	1.73^{c}	1.69^{c}	0.039	0.07	<.001	0.32
	15-20 d	2.08^{a}	1.9^{ab}	1.75^{b}	1.79^{b}	0.05	2.13^{a}	1.89^{b}	1.79^{b}	1.7^{b}	0.05	<.001	<.001	0.4
	20-25 d	2.03^{a}	1.91^{ab}	1.76^{b}	1.73^{b}	0.063	2.00^{a}	1.87^{ab}	1.82^{ab}	1.74^{b}	0.063	<.001	0.03	0.03
	25-30 d	2.05^{a}	1.91^{ab}	1.89^{ab}	1.84^{b}	0.051	2.04^{a}	1.95^{ab}	1.84^{b}	1.86^{ab}	0.051	0.023	0.034	0.77
	30-35 d	2.11^{a}	2.07^{ab}	1.96^{ab}	1.89^{b}	0.054	2.15^{a}	2.00^{ab}	1.92^{b}	1.96^{ab}	0.054	0.022	0.02	0.94
	5-35 d	1.98^{a}	1.86^{b}	1.8^{bc}	1.77^{c}	0.02	2.01^{a}	1.87^{b}	1.77^{c}	1.76^{c}	0.02	<.001	<.001	0.42

$^{a, b, c}$ Means within each row with uncommon superscript are significantly different (P< 0.05)

Table 3: Effect of various levels of energy and protein on relative organ weights of broiler chicks at days 10, 15 and 20 of age

	ME					CP					Probability		
	2900	3000	3100	3200	SEM	17	20	23	26	SEM	ME	CP	ME*CP
10 d of age													
LBW (g/b)	175.6	173.4	191.9	182.7		174.4	183.2	182.3	183.7				
	(% of Live Body Weight)												
Liver	4.21	4.23	4.25	4.2	0.136	4.79^{a}	4.00^{b}	4.08^{b}	4.01^{b}	0.136	0.952	<0.01	0.685
Spleen	0.096	0.089	0.107	0.087	0.008	0.082	0.105	0.088	0.105	0.008	0.48	0.058	<0.01
Bursa	0.16	0.185	0.17	0.19	0.01	0.163	0.170	0.176	0.197	0.01	0.14	0.12	0.025
15 d of age													
LBW (g/b)	297	339	308.5	324.2		289.8	318.0	331	329.2				
	(% of Live Body Weight)												
Liver	3.44	3.36	3.37	3.44	0.10	3.52	3.24	3.40	3.44	0.10	0.89	0.13	0.6
Spleen	0.107	0.085	0.086	0.094	0.008	0.091	0.084	0.104	0.03	0.008	0.22	0.28	0.75
Bursa	0.238	0.207	0.203	0.213	0.013	0.225	0.215	0.203	0.217	0.013	0.18	0.65	0.83
20 d of age													
LBW (g/b)	451.1	497.3	483.1	504.0		430.6	505.0	512	518.2				
	(% of Live Body Weight)												
Liver	3.18	3.25	3.04	3.44	0.126	3.66^{a}	3.20^{ab}	3.03^{b}	3.02^{b}	0.126	0.17	0.003	0.70
Spleen	0.115	0.107	0.113	0.118	0.008	0.109	0.114	0.123	0.107	0.008	0.59	0.35	0.42
Bursa	0.24	0.23	0.24	0.232	0.013	0.235	0.234	0.236	0.24	0.013	0.97	0.93	0.84

$^{a, b,}$ Means within each row with uncommon superscript are significantly different (P< 0.05)

Table 4: Effect of various levels of ME and CP on total anti-SRBC, IgG and IgM titers of broiler chickens, 5 and 10 days after the first and second SRBC injection[1]

		Days PPI[2]						Days PSI[3]					
		5			10			5			10		
Sources	Level	Total anti-SRBC	IgG	IgM	Total anti-SRBC	IgG	IgM	Total anti-SRBC	IgG	IgM	Total anti-SRBC	IgG	IgM
ME	2900	5.61^{a}	4.25^{ab}	1.360	3.60^{a}	3.33^{a}	0.270	7.410	6.150	1.260	4.860	4.65^{a}	0.210
	3000	5.26^{ab}	4.50^{a}	0.760	3.07^{a}	2.77^{ab}	0.300	6.990	6.270	0.720	4.560	4.18^{a}	0.380
	3100	5.38^{ab}	4.25^{ab}	1.130	3.40^{a}	3.31^{a}	0.090	6.590	5.780	0.810	4.350	4.25^{ab}	0.100
	3200	4.66^{b}	3.70^{b}	0.960	2.45^{b}	2.26^{b}	0.190	6.770	6.570	1.100	4.110	3.78^{b}	0.330
	SEM	0.240	0.199	0.249	0.166	0.183	0.156	0.278	0.313	0.245	0.206	0.162	0.145
CP	17	5.420	4.40	1.020	3.260	2.930	0.330	7.000	6.140	0.860	4.370	4.11	0.260
	20	5.310	4.09	1.220	3.160	2.810	0.330	6.830	6.170	0.660	4.460	4.26	0.200
	23	5.190	4.30	0.900	2.990	2.800	0.190	7.110	6.060	1.050	4.650	4.33	0.320
	26	4.980	3.93	1.050	3.130	3.000	0.130	6.830	5.510	1.320	4.420	4.17	0.250
	SEM	0.240	0.199	0.249	0.166	0.183	0.156	0.278	0.313	0.245	0.206	0.162	0.145
Source of variation		Probability											
ME		0.044	0.045	0.385	<.001	<.001	0.763	0.190	0.162	0.360	0.079	<.001	0.311
CP		0.598	0.337	0.826	0.757	0.524	0.324	0.865	0.258	0.272	0.780	0.86	0.961
ME×CP		0.563	0.009	0.388	0.074	0.375	0.363	0.170	0.840	0.692	0.396	0.711	0.470

^{a,b} Means within a column and classification with no common superscript differ significantly ($P \leq 0.05$)

[1]Four birds per pen were injected by SRBC (10% suspension in PBS, 1 ml/chick) at 15 and 26 day of age. Blood serum samples in all birds per treatment were analyzed for the presence of total anti-SRBC, IgG and IgM antibodies. The data represent mean of log2 of the reciprocal of the last dilution exhibiting agglutination

[2]days postprimary injection [3]days postsecondary injection

results of current study. Praharaj *et al.* [4] reported that the developments of immune organs in broiler chicks (Bursa of Fabricius and spleen) were not influenced by dietary energy levels (2,500, 2,650 and 2,800 kcal/kg of ME) from 1 to 42 d. In study by Liu *et al.* [32], the relative weight of liver was greater for chickens fed diet included 20% protein and 11.23 MJ/kg energy than those fed diet contained 24% protein and 13.16 MJ/kg energy. Our results are consistent with Namroud *et al.* [33] results who accounted a decrease in relative liver weight with decreasing of diet crude protein (17%). Simultaneously, they observed increased blood ammonia level and decreased plasma uric acid when chicks fed diet contained 17% CP. They concluded that increasing of blood ammonia level activates some mechanisms, one of which (conversion of ammonia to uric acid) mainly occur in the liver. Therefore, the increasing weight of liver might be due to adaptation to elevated ammonia production, but further work is needed to detect enzymes activity associated with uric acid synthesis, especially xanthine oxidase. As result show, low BW may be due partly to the increasing activities of enzymes involved in uric acid production and subsequently extra liver growth.

Immune Responses: The effect of various levels of energy and protein on total, IgG and IgM antibody titers is shown in Table 4. Total anti-SRBC titers were linearly increased ($P<0.05$) in birds fed low energy diets at day's postprimary (PPI) but not in postsecondary injections (PSI) days. A similar result was observed for IgG at day's PPI and at d 10 PSI ($P <0.05$). Immunoglobulin M titers were not significantly changed by different levels of energy that measured at PPI and PSI ($P<0.05$). Protein levels did not have any significant effect on total anti-SRBC, IgG and IgM antibody titers at PPI and PSI ($P>0.05$). The energy by protein interaction was not observed for total, IgG and IgM antibody titers at PPI and PSI ($P>0.05$) except at d 5 PPI ($P=0.009$).

The present results corroborate Praharaj *et al.* [4] reports that the response to SRBC inoculation of broilers fed a diet with a lower nutrient density was equal or greater to those fed a diet with a high nutrient density. Korver *et al.* [34] found that the low energy diet (2700 kcal/kg) resulted in the poorest performance than high energy diet (3300 kcal/kg), but it did not influence on chicks immune response which is in contrast to our results. Also, Praharaj *et al.* [4] fed commercial broilers diets differing in energy (2,500, 2,650 and 2,800 kcal/kg of ME) from 1 to 42 d of age found no differences in primary antibody response to SRBC. Liu *et al.* [32] did not find any difference in antibody titers to SRBC in chicks fed diet included 20% protein and 11.23 MJ/kg energy to those fed diet contained 24% protein and 13.16 MJ/kg.

Increasing level of energy and protein in broiler diets usually improved growth rate. There are negative phenotypic relations between immunocompetence and rapid growth in chickens [35-37]. Also; there is a positive correlation between genetic changes with macrophage phagocytic activity [38]. Additional studies in chickens [18, 39, 40] have supported the current conclusions that the antibody responses and disease resistance potential of fast growing strains of poultry is relatively weaker than in the slower growing types from which they are selected. There is a need for high humoral immune responses in birds for protecting against diseases which one strategy is the application of DNA markers in indirect selection for quantitative traits, such as immune response and disease resistance [41-43]. Broiler chickens are reaching market weights at an earlier age and it has been estimated that approximately 85 to 90% of this rapid growth is due to genetic selection by the primary breeders [2]. On the other hand, that is an interest in diet formulation to find the best level of energy and protein to improve humoral immune response as well as growth. Dietary characteristics can modulate a bird's humoral immune response and subtle influences due to the levels of nutrients or the types of ingredients may at times be of critical importance.

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