Glucose and Electrolyte Supplementation of Drinking Water Improve the Immune Responses of Poults with Inanition[1]

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ABSTRACT Enteric disorders predispose poultry to malnutrition. The objectives of this paper were 1) to simulate the inanition of poult enteritis mortality syndrome by restricting feed intake and 2) to develop a drinking water supplement that supports the immune functions of poults with inanition.

Poults were restricted to 14 g of feed/d for 7 d beginning at 14 d of age then fed ad libitum until 36 d (recovery). The control was fed ad libitum. During the feed-restriction period, duplicate groups of 6 poults received 1 of 5 drinking water treatments: 1) restricted feed, unsupplemented water; 2) restricted feed + electrolytes (RE); 3) RE + glucose + citric acid (REGC); 4) REGC + betaine (REGCB); or 5) REGCB + zinc-methionine (REGCBZ). Immunological functions were assessed by inoculating poults with SRBC and *B. abortus* (BA) antigen at 15, 22, and 29 d of age. Antibody (Ab) titers were determined 7 d later for primary, secondary, and recovery responses. The primary and secondary total Ab titers to SRBC for restricted feed were 4.71 and 6.16 $\log_3$, which where lower ($P < 0.05$) than for controls (8.00 and 9.66 $\log_3$) and the other treatments. The recovery Ab titer for controls was 10.7, significantly higher than restricted feed (8.71) and RE (8.10) groups but not different from other treatments. The primary total Ab responses to BA were significantly lower in the restricted feed and RE groups as compared with the control and other treatments. Although feed restriction of poults to maintenance reduces the humoral immune responses, these responses can be significantly improved by drinking water containing electrolytes and especially sources of energy such as glucose and citric acid.

(*Key words*: electrolyte, energy, feed restriction, glucose, immune response)

INTRODUCTION

Poult enteritis and mortality syndrome (PEMS) is an acute multifactorial disease with a rapid onset and relatively short duration as compared with some other enteric disorders. Several microbes have been implicated as etiological agents of PEMS (Edens et al., 1998; Yu et al., 2000). PEMS occurs during the second and third weeks after hatching and is characterized by initial vocalization from the poults, feed refusal, diarrhea, weight loss, immune dysfunction, and a mortality rate of 20 to 50% (Barnes and Guy, 1997; Qureshi et al., 1997). Feed refusal and inanition are common clinical signs of PEMS, which lead to hypoglycemia and hypophosphatemia (Edens et al., 1998). Usually, there is little or no body weight gain during the 8- to 13-d course of the active disease. Average feed intake is sufficient only to maintain body weight (K. Krueger, 1997, Diamond K, Marshville, NC, personal communication). PEMS survivors have stunted growth and are not capable of complete compensatory growth based on the definition of accelerated growth (Odetallah et al., 2001). Consequently, among PEMS survivors, there is great variation in BW at market age. The high mortality associated with PEMS may be attributed primarily to dehydration and hypothermia from lack of dietary electrolytes, water, and energy. Studies with human infants, calves, and piglets afflicted with acute enteric disease have shown that mortality can be reduced significantly by oral administration of physiologically balanced solu-

Abbreviation Key: Ab = antibody; BA = *Bacillus abortus*; PEMS = poult enteritis mortality syndrome; PEP = poultry electrolyte preparation; RE = feed restricted + electrolytes; REGC = feed restricted + electrolytes + glucose + citric acid; REGCB = feed restricted + electrolytes + glucose + citric acid + betaine; REGCBZ = feed restricted + electrolytes + glucose + citric acid + betaine + zinc methionine; R = feed restricted + water.

tions of water, electrolytes, and glucose (Hirschhorn, 1982; Wilson et al., 1990). Furthermore, severe malnutrition impairs the immune response of humans and poultry (Nathan et al., 1977; Klasing, 1988). Preliminary studies with turkey poults have established that the absolute and relative weights of the immune organs are significantly decreased when poults are restricted to maintenance feed intake (El-Hadri et al., 1998).

With respect to turkey poults, there is limited knowledge of immune responses to malnutrition or to nutritional therapy via drinking water. Therefore, the objectives of the present study were to determine 1) the degree of feed restriction needed to achieve body weight maintenance in noninfected poults and 2) to determine the effect of nutrients (electrolytes, glucose, betaine, and zinc-methionine) supplied in the drinking water on the immune response of feed-restricted poults.

MATERIALS AND METHODS

Two trials with poults were conducted under a protocol approved by the Institutional Animal Care and Use Committee of North Carolina State University (Federation of Animal Science Societies, 1999). Day-old female Nicholas turkey poults were obtained from a commercial hatchery and grown to 15 d of age in wire-bottom cages in an animal-care facility with thermostatically controlled environmental temperature (32°C). The poults consumed a nutritionally complete turkey starter diet (Table 1) and water ad libitum. During the first 5 d of age, the poults were given a fluoroquinolone antibiotic (30 mg/L) in drinking water to eliminate potential enteric pathogens (e.g., *Salmonella*). Preliminary experiments revealed that feed restriction increased the susceptibility of poults to latent opportunistic bacterial infection, such as salmonellosis (unpublished data). At 10 d of age the poults were weighed individually and randomly assigned to their respective treatment groups. The poults were weighed again at 15 d of age when the 2-wk treatment period began.

Trial 1

The control group consumed feed ad libitum throughout the experiment. From 15 to 29 d of age, treatment groups of poults were progressively restricted in feed intake to determine the maintenance level of feed consumption, a situation similar, but not identical, to the inanition associated with PEMS. Feed intake was restricted to 10, 12, 14, and 16 g/d per poult for 2 wk. The poults were fed once per day. Water was consumed ad libitum. Body weights were recorded at 15, 22, and 29 d of age. Each group was represented by 2 replicate pens of 6 female poults per pen.

[5]Zinpro 200, Zinpro Corporation, Eden Prairie, MN.

TABLE 1. Composition of Turkey Starter Diet

Ingredient	Weight (kg)
Soybean meal, 48.5% CP	500.0
Corn, 8.25% CP	427.8
Poultry fat	20.0
NaCl	2.0
NaHCO$_3$	4.0
Selenium premix (0.02%)	1.0
CaHPO$_4$·2H$_2$O	25.0
Limestone	14.5
DL-Methionine	2.1
Ethoxyquin	0.1
L-Lysine HCl	1.5
Trace mineral premix[1]	2.0
Vitamin premix[2]	1.5
Total	1,001.5
Calculated nutrient content	
ME (kcal/kg)	2,850.0
CP (%)	28.02
Fat (4%)	2.00
Methionine + cysteine	1.12
Methionine	0.66
Lysine	1.83
Threonine	1.13
Arginine	2.07
Tryptophan	0.37
Histidine	0.75
Isoleucine	1.41
Leucine	2.34
Phenylalanine	1.50
Valine	1.52
Linoleic acid	1.60

[1]The trace mineral premix provided the following (mg/kg of diet): Zn, 120; Mn, 120; Fe, 80; Cu, 10; Co, 1; sulfate, 560; iodine, 2.5, as iodate.

[2]The vitamin premix provided the following (mg/kg of diet): vitamin A, 6.8; vitamin D$_3$, 0.15; vitamin E, 99; vitamin B$_{12}$, 0.06; riboflavin, 20; niacin, 165; D-pantothenate, 33; menadione, 6; folic acid, 3.3; thiamine, 6.0; pyridoxine, 12; D-biotin, 0.38.

Trial 2

Two-week-old female poults were allotted to six treatments. In treatment 1, the control birds consumed feed ad libitum. In treatments 2 through 6, birds were restricted to 14 g feed/poult per day for maintenance of body weight and given 1 of 5 water-supplemented treatments: water (R), R + electrolytes (RE), RE + glucose and citric acid (REGC), REGC + betaine (REGCB), and REGCB + zinc methionine (REGCBZ). Each of the 6 treatment groups was randomly assigned to 3 replicate pens of 6 birds/pen. The formulation of the poultry electrolyte preparations (PEP) was based on the oral rehydration solution recommended by the World Health Organization (1980). Glucose and citric acid served as sources of energy. Citric acid lowered the pH of the solution to 3, which retarded microbial growth. Addition of betaine served as an osmolyte, methyl donor, and source of glycine (Krumdieck, 1990; Ferket, 1995; Garlich, 1995). Betaine has been shown to reduce the severity of diarrhea in turkeys with "flushing" syndrome (Ferket, 1996; Ferket and Veldkamp, 1999). Zinc-methionine[5] was added to the solution at 42 μg/mL (8.4 μg of Zn/mL) because it has been reported to stimulate the immune system (Kidd et al., 1996).

The complete PEP formulation, EGCBZ, is shown in Table 2. The solutions were prepared daily and made

Ingredient	g/L
NaCl	1.65
NaHCO$_3$	0.263
KH$_2$PO$_4$	2.70
Glucose, monohydrate	19.00
Citric Acid	4.60
Betaine, free base	3.00
Zinc-methionine[3]	0.042
Calculated composition	(M)
Na, mEq/L	31
Cl, mEq/L	28
K, mEq/L	20
Pi, mmol/L	20
Citrate, mmol/L	22
Glucose, mmol/L	96
Betaine, mmol/L	25.4
Zinc, mg/L	8.4
Energy, kcal/L	83
Osmolarity, mOsm/L	242
pH	3.0

[1]The Na and Cl conform to suggestions (Dibley et al., 1984) regarding use of hypotonic oral solutions for the prevention of dehydration.

[2]The osmolarity is below the limits (360 mOsm) suggested by the World Health Organization (Hirschhorn, 1982).

[3]Zinc-methionine contained 20% zinc and was provided in the form of Zinpro-200 by Zinpro Corporation (Eden Prairie, MN).
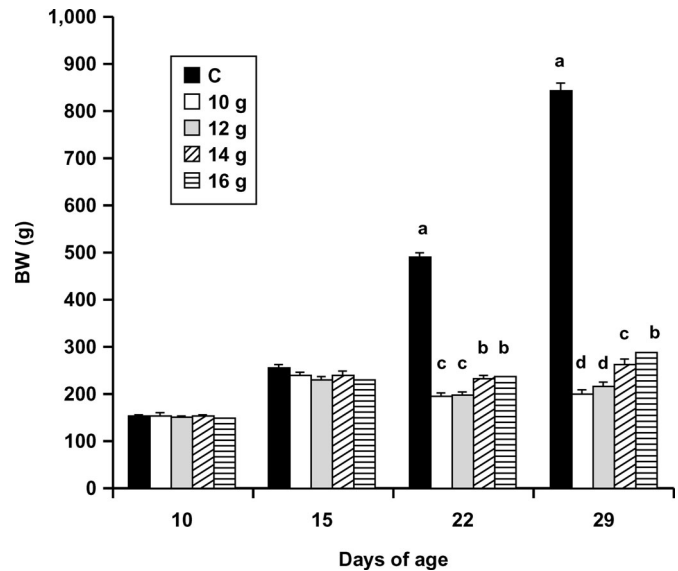

FIGURE 1. Effect of different levels of feed restriction on body weight (g) during 2 wk of feed restriction (15 to 29 d of age). Feed was restricted to 10, 12, 14, or 16 g/d per poult. Control birds (C) were provided feed ad libitum. Values represent means of 2 pens of 6 birds per pen for each treatment group. Bars represent means ± standard error of the mean. [a–c]Bars within the same age with differing superscripts are significantly different ($P < 0.05$).

available ad libitum in 700-mL drinking jars. Fluid consumption for each pen was recorded daily. The average consumption per poult per pen was determined.

Two weeks of feed restriction were followed by a recovery period of 1 wk, during which the poults consumed feed and water ad libitum. Body weights were recorded at 15, 22, and 29 d of age.

Immunological Assessment

The washed SRBC,[6] a T-cell-dependent antigen (McArthur et al., 1973), and *Brucella abortus*[7] (BA), a T-cell-independent antigen (Gilmour et al., 1970), were used to assess the humoral immune response. The killed BA (10% original concentration) was then diluted 10 times with PBS (pH 7.4) containing 3.5% bovine serum albumen.

Immunization

The BA and washed SRBC suspensions (7% in PBS) were mixed together in 1:6 (vol/vol) ratio of BA:SRBC suspension (Qureshi et al., 1998). One milliliter of this suspension was given i.v. via the jugular vein at 15 d age (onset of feed restriction), at 22 d of age (1 wk of feed restriction), and at 29 wk of age (2 wk of feed restriction). Blood samples were taken 7 d after each immunization.

[6]Small Animal Unit, North Carolina State University, Raleigh, NC.
[7]Rollins Veterinary Diagnostic Laboratory, North Carolina Department of Agriculture, Raleigh, NC.

Hemagglutination Assay

The BA and SRBC antibody (Ab) titers were determined in the serum of each poult by the microtiter procedure of Wegman and Smithies (1966) and McCorkle and Glick (1980). The titers were recorded as total antibodies expressed as log$_3$.

Statistics

The experimental unit was a pen of 6 birds for both trials. Data were subjected to analysis of variance using the GLM procedures of SAS software (SAS Institute, 1996). Least-squares differences were used to determine the significance between means. Statements of statistical significance are based on $P \leq 0.05$ unless otherwise indicated.

RESULTS

Trial 1

Figure 1 illustrates the effect of different levels of feed restriction on body weight from 15 to 29 d of age. Poults restricted to 10 or 12 g of feed/d lost weight during this 14-d period, whereas those receiving 14 or 16 g/d gained an average of 7 or 41 g, respectively. Based on these results, the poults of trial 2 were restricted to 14 g of feed per day per poult (i.e., for maintenance of body weight but no growth) to simulate the level of feed intake typically observed among PEMS-afflicted poults. This maintenance level represents 21% of ad libitum feed intake. No mortality was observed during the 2 wk of feed restriction.
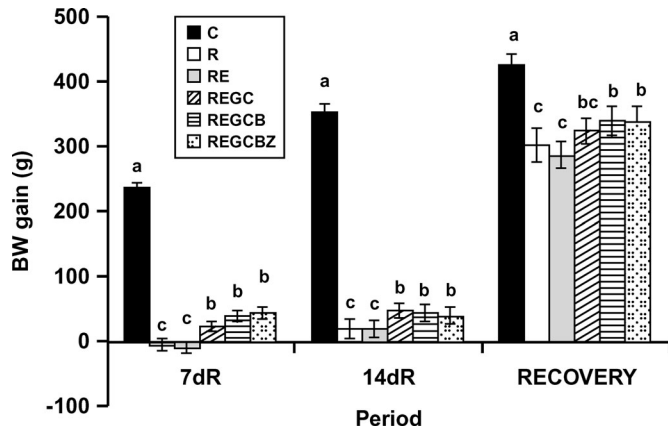
FIGURE 2. Effect of poultry electrolytes preparation (PEP) on body weight gain (g) at 7 d of feed restriction (22 d of age, 7dR) and at 14 d of feed restriction (29 d of age, 14 d R). R = feed restricted + water; RE = feed restricted + electrolytes; REGC = feed restricted + electrolytes + glucose + citric acid; REGCB = feed restricted + electrolytes + glucose + citric acid + betaine; REGCBZ = feed restricted + electrolytes + glucose + citric acid + betaine + zinc methionine. Bars represent means ± standard error of the mean of 3 pens of 6 birds per pen for each treatment group. [a-c]Bars within the same period with differing superscripts are significantly different ($P < 0.05$).

Trial 2

Poults restricted to 21% of the feed consumed by the control group fed ad libitum had ($P \leq 0.0001$) lower BW after the 2-wk period of feed intake restriction (Figure 2). Among the feed-restricted poults, those that consumed drinking water containing an energy supplement of glucose and citric acid had greater body weights ($P \leq 0.05$) than those that consumed water only (R) or electrolytes only (RE).

Water consumption of the control poults fed ad libitum was not determined. The average fluid consumptions of the feed-restricted poults were 36, 81, 120, 123, and 127 mL/poult per day for treatments R, RE, REGC, REGCB, and REGCBZ, respectively (Figure 3). The supplementa-
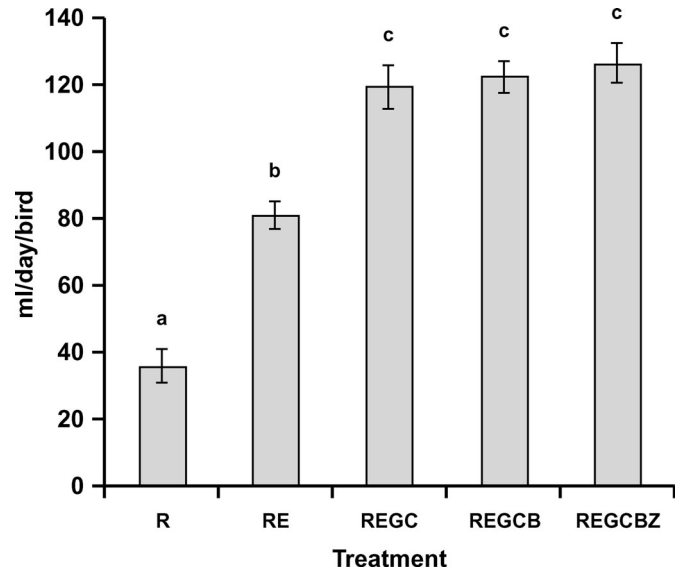
TABLE 3. Antibody response against SRBC[1]

Treatment[2]	Primary	Secondary	Tertiary (recovery)
		(log$_3$)	
Control	8.00[a]	9.66[a]	10.71[a]
R	4.71[b]	6.16[b]	8.71[b]
RE	7.27[a]	7.75[ab]	8.10[b]
REGC	7.58[a]	8.66[a]	9.27[ab]
REGCB	7.00[a]	9.66[a]	9.80[ab]
REGCBZ	6.28[a]	9.48[a]	9.30[ab]

[a,b]Means in the same column not followed by the same superscript letter are significantly different, $P < 0.05$.

[1]Poults were inoculated i.v. at 15, 22, and 29 d of age. Antibody titers (log$_3$) were determined 7 d after inoculation. All groups restricted to maintenance feed intake (R; 14 g/d per poult) from 15 to 29 d of age were provided feed ad libitum from 29 to 36 d of age (recovery).

[2]Control = fed ad libitum; R = feed restricted + water; RE = feed restricted + electrolytes; REGC = feed restricted + electrolytes + glucose + citric acid; REGCB = feed restricted + electrolytes + glucose + citric acid + betaine; REGCBZ = feed restricted + electrolytes + glucose + citric acid + betaine + zinc methionine.

FIGURE 3. Average fluid consumption per poult per day during 2 wk of feed restriction. R = feed restricted + water; RE = feed restricted + electrolytes; REGC = feed restricted + electrolytes + glucose + citric acid; REGCB = feed restricted + electrolytes + glucose + citric acid + betaine; REGCBZ = feed restricted + electrolytes + glucose + citric acid + betaine + zinc methionine. Bars represent means ± standard error of the mean for water and PEP consumption of 3 pens of 6 birds per treatment group. [a-c]Bars within the same period with differing superscripts are significantly different ($P < 0.05$).

tion of drinking water with electrolytes and glucose greatly increased fluid consumption among the feed-restricted poults (36, 81, and 123 mL/poult for the R, RE, and REGC treatments, respectively). Energy supplementation in the drinking water resulted in increased body weight by 70 or more g/poult during the 2 wk of feed restriction, whereas the controls fed ad libitum gained 630 g (data not shown). There was no mortality in the control group. However, mortality rate was 17, 5, 0, 11, and 22% for the R, RE, REGC, REGCB, and REGCBZ, respectively.

During the 1-wk recovery period (29 to 36 d of age), poults that had received only water (R) or electrolytes (RE) had average body weight gains of 302 and 286 g/bird, respectively, whereas those that received the supplemental energy from glucose and citric acid gained 324, 339, and 338 g/bird (RG, RGCB and RGCBZ, respectively) (Figure 2).

Immune Responses

Table 3 illustrates treatment effects on primary, secondary, and recovery Ab response to SRBC inoculation after 7 and 14 d of feed restriction and 7 d after re-alimentation (recovery). In general, total Ab titers increased after the second and third inoculations of SRBC. The primary, secondary, and recovery titers for total Ab to SRBC were significantly lower in the feed-restricted poults receiving only water (R) as compared with the control poults fed ad libitum. In contrast, the feed-restricted poults that received electrolytes plus glucose (REGC, REGCB, and

Treatment[2]	Primary	Secondary	Tertiary (recovery)
		(log$_3$)	
Control	5.88[a]	7.22[a]	8.50[a]
R	2.33[b]	2.57[d]	5.60[b]
RE	3.50[b]	3.11[cd]	7.41[a]
REGC	5.25[a]	4.22[bc]	7.16[a]
REGCB	5.66[a]	4.00[bc]	6.50[b]
REGCBZ	5.33[a]	4.37[b]	7.33[a]

[a–d]Means in the same column not followed by the same superscript letter are significantly different, $P < 0.05$.

[1]Poults were inoculated i.v. at 15, 22, and 29 d of age. Antibody titers (log$_3$) were determined 7 d after inoculation. All groups restricted to maintenance feed intake (R; 14 g/d per poult) from 15 to 29 d of age were provided feed ad libitum from 29 to 36 d of age (recovery).

[2]Control: fed ad libitum; R = feed restricted + water; RE = feed restricted + electrolytes; REGC = feed restricted + electrolytes + glucose + citric acid; REGCB = feed restricted + electrolytes + glucose + citric acid + betaine; REGCBZ = feed restricted + electrolytes + glucose + citric acid + betaine + zinc methionine.

REGCBZ) exhibited Ab titers that were significantly greater than restricted feed birds and not different from control poults. The response to electrolytes, RE, was significantly better than restricted feed only for the primary response.

Table 4 illustrates the treatment effects on primary (7 d) and secondary (14 d) Ab response to BA inoculation after 7 and 14 d of feed restriction and subsequently 7 d after re-alimentation (recovery). In comparison to the restricted feed and RE treatment groups, poults that consumed energy from glucose and citric acid (REGC, REGCB, and REGCZ) via drinking water showed significantly higher primary Ab titers to BA. Recovery Ab titers indicated a possible benefit to electrolytes alone (RE). Addition of betaine or zinc-methionine to drinking water containing electrolytes and glucose did not produce higher Ab titers to SRBC nor to BA antigens.

DISCUSSION

Restricting feed intake to maintenance (14 g/d per poult, or about 40 calories/d) in noninfected poults from 14 to 29 d of age suppressed the thymic-dependent and thymic-independent immune responses as indicated by a significant decrease in the Ab response against SRBC and BA antigens. This suppression in humoral immune response was significantly alleviated when the restricted poults consumed drinking water supplemented with electrolytes and energy sources of glucose and citric acid. The additional energy intake was sufficient to produce a slight increase in body weight gain of about 5 g/poult per day. Such positive response might be explained by the increased intake of energy (primarily glucose) via the drinking water. This additional energy appears to have spared dietary protein (i.e., increased the efficiency of utilization of dietary protein for growth). Increased consumption of fluids containing glucose appeared to be an attempt of the growth-stunted poults to fulfill their needs for energy. Kaunitz et al. (1995) and Nappert et al. (2000)

reported that a glucose-based solution stimulates intestinal absorption of electrolytes from isotonic luminal contents. This finding suggests that the use of PEP, containing glucose and citric acid, may restore solute and volume deficits that result from diarrhea and provide energy to anorexic birds. Similarly, Hirschhorn (1982) reported that treatment with an oral rehydration solution containing electrolytes and glucose alleviates anorexia and stimulates food intake in human infants with diarrhea.

In the case of PEMS, greatly reduced voluntary feed intake, poor absorption, and hypophosphatemia (phosphate reserves become depleted) as a consequence of the disease agents result in a spike in mortality rate (Edens, 1994). When they begin to consume feed again, the high demand for phosphate to phosphorylate the incoming glucose reduces the blood inorganic phosphate to fatally low levels (Ferket, 1995). Edens and Doerfler (1997a,b) also reported that glucose metabolism in PEMS-infected poults appears to be impaired because hepatic glycogen is depleted, and hepatic glucose-6-phosphatase activity is increased without concomitant increases in serum glucose. Even with addition of sucrose to the drinking water of infected poults, these conditions were not corrected (Edens and Doerfler, 1997a). It is important to point out here that poults used in this study did not have PEMS. Therefore, the former consequences may not apply to our feed restriction model in which poults were restricted in feed to maintenance in order to simulate the situation observed in PEMS reported by K. Krueger (1999, personal communication).

Our results are supported by the works of Kaunitz et al. (1995) and Nappert et al. (2000). They found that the immune response is compromised by malnutrition but can recover if sufficient energy and electrolytes are supplied via drinking water. Nathan et al. (1977) reported that antibody response in chicks fasted for 24 or 48 h are impaired. In a comprehensive literature review, Chandra (1994) described immune dysfunctions in infants with protein-energy malnutrition. In the malnourished humans (Ozkan, 1993), nutrient deficiencies altered either cellular or humoral immune mechanisms and increased the susceptibility to bronchopneumonia and gastroenteritis infections. Klasing (1998) proposed that a threshold nutrient sufficiency is required for the initial development of the immune response so that responding cells can divide and synthesize effector molecules. Our results support this hypothesis and demonstrate that additional energy, sufficient to increase body weight gain by 5 g/poult per day, significantly enhanced the humoral immune response. Electrolytes alone had a modest although significant effect on stimulating the humoral immune response to SRBC without affecting the body weight gain.

In the present work, addition of betaine and zinc-methionine to solutions already providing electrolytes and glucose did not increase titers to SRBC and BA. The lack of response to betaine may be due to the fact that its osmorugulating properties were not needed in this feed restriction situation. This assumption is supported by several experimental findings. For example, renal cells have

an active sodium-dependent betaine transport that increases in activity under osmotic stress in vitro (Nakanishi et al., 1990). Furthermore, Madin-Darby canine kidney cells accumulate betaine and other osmolytes when cultured in hypertonic medium (Nakanishi et al., 1988, 1990). In infectious diarrheal conditions caused by bacteria (e.g., *Clostridium*, *Escherichia*, or *Salmonella*) and viruses (e.g., adenovirus, coronavirus, or rotavirus) adenylate cyclase, and cAMP activities are increased and stimulate crypt cell secretory activity and decrease villous absorption of sodium chloride (Fondacaro, 1986) leading to hyperosmolar medium in the intestinal lumen (Cantey, 1993). Such hyperosmolar environment may not be present in our noninfected, feed-restricted poults.

With the addition of zinc-methionine to the PEP, we hypothesized an increase in thymic-dependent antigens (e.g., to SRBC). This hypothesis was based on thymic defects and thymic atrophy being more pronounced in progeny from dams that received inadequate dietary zinc (Beach et al., 1982). Zinc is associated with the thymic hormone thymulin (Dardenne and Bach, 1993). Supplementation of zinc-methionine in turkey diets significantly increased immune response in turkeys with enteritis (Kidd et al., 1994). However, no positive effects of zinc-methionine were observed in our PEMS model.

Based on our results we suggest that poults and chicks with acute enteritis would benefit from drinking water solutions containing a balanced electrolyte mixture and glucose plus citric acid as sources of energy. Furthermore, the osmolarity of the solution should be low enough (250 mOsm or less) so that it does not inhibit feed intake (Garlich, 1997) or cause hypernatremia in birds that are not as severely affected by the disease (Nappert et al., 2000). Inclusion of citric acid produces a solution of pH 3, which retards microbial growth in the watering system. Further, formulation of PEP must take into consideration the pathological processes (invasive or toxigenic) and diarrheal mechanisms (osmotic or secretory). Other critical nutrients should be investigated.

In conclusion, turkey poults, whose feed intake is restricted to maintenance (no growth) from 14 to 29 d of age (a situation observed with acute enteritis), exhibit impaired antibody production. Supplementing the intakes of these poults with critical nutrients in the drinking water significantly improved antibody production. The use of an electrolyte solution alone had a modest effect on T-cell-dependent antibody production. However, adding sources of readily available energy (e.g., glucose plus citric acid) significantly improved antibody responses to the T-cell-dependent (SRBC) and T-cell-independent (BA) antigens while increasing body weight gain to a modest 5 g/poult per day. Addition of betaine and zinc-methionine to this solution had no effect on the humoral immune responses in this model.

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