Revista Brasileira de Zootecnia
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ISSN 1806-9290
www.sbz.org.br

R. Bras. Zootec., v.39, n.1, p.165-174, 2010

Yeast extract and prebiotic in pre-initial phase diet for broiler chickens raised under different temperatures[1]

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ABSTRACT - The objective of this research was to evaluate the performance, carcass yield and intestinal morphometry of broiler chickens raised under different temperatures that received feed with or without yeast extract and prebiotic in the pre-initial phase. One thousand four hundred and forty one-day old male chicks were used, raised in different climate chambers. Feed with or without the addition of yeast extract and prebiotic was offered only in the pre-initial phase (1 to 7 days). From the eighth day on, every chick received the same feed, readjusted according to usual recommendations. A randomized complete experimental design was used in a 3 × 2 × 2 factorial arrangement, consisting of three environmental temperatures (hot, comfort and cold) and two levels of yeast extract (with or without) and prebiotic (with or without). The performance of the birds was evaluated considering weight gain, feed intake, food conversion and viability at 42 days of age. Carcass yield and intestinal morphometry were also evaluated. Environmental heat impaired performance and carcass yield. Prebiotic inclusion in the pre-initial feed increased weight gain and enhanced food conversion of birds raised under hot conditions. The inclusion of products in the feed of broiler chickens raised in hot and cold environments has beneficial effects on chicken intestinal villi.

Key Words: carcass yield, heat stress, intestinal morphometry, mannanoligosaccharides, performance, *Saccharomyces cervisiae*

Extrato de leveduras e prebiótico na dieta pré-inicial de frangos de corte criados em diferentes temperaturas

RESUMO - Objetivou-se nesta pesquisa avaliar o desempenho, o rendimento de carcaça e a morfometria intestinal de frangos de corte criados em diferentes temperaturas e que receberam na fase pré-inicial ração contendo ou não extrato de leveduras e prebiótico. Foram utilizados 1.440 pintos machos de 1 dia de idade, criados em diferentes câmaras climáticas. As rações, acrescidas ou não de extrato de leveduras e prebiótico, foram oferecidas somente na fase pré-inicial (1 a 7 dias). A partir do oitavo dia, todas as aves receberam a mesma ração, reajustada de acordo com as recomendações usuais. Adotou-se o delineamento experimental inteiramente casualizado em arranjo fatorial 3 × 2 × 2, composto de três temperaturas de criação (calor, conforto e frio) e dois níveis de extrato de leveduras (com ou sem) e prebiótico (com ou sem). O desempenho das aves foi avaliado considerando o ganho de peso, o consumo de ração, a conversão alimentar e a viabilidade aos 42 dias de idade. Também foram avaliados o rendimento de carcaça e a morfometria intestinal. O calor ambiente prejudicou o desempenho e o rendimento de carcaça. A inclusão de prebiótico na ração pré-inicial aumentou o ganho de peso e melhorou a conversão alimentar das aves criadas no calor. A inclusão dos produtos na ração de frangos de corte criados em ambiente de calor e no frio tem efeito benéfico sobre as vilosidades das aves.

Palavras-chave: desempenho, estresse calórico, mananoligossacarídeos, morfometria intestinal, rendimento de carcaça, *Saccharomyces cerevisiae*

Introduction

The use of diversified diet for broiler chickens in the first week of age has been recommended by several nutritionists. This practice is justified by the fact that broiler chickens at that age have specific and different nutritional needs than in other phases, probably due to different gastrointestinal tract characteristics and their difficulty in digesting and absorbing some nutrients because of the rapid development. Thus, substances that

Received March 24, 2008 and accepted April 27, 2009.
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have trophic action on intestinal mucosa may enhance the performance of chickens and provide better capacity for digesting and absorbing nutrients from the diet.

Yeast extract is a protein source derived from the cell content of live yeast. It is rich in nucleotides, inositol and glutamic acid. Nucleotides are used in human nutrition, mainly in diets for newborns, and act on gastrointestinal development, imune system functioning and intestinal flora maintenance. Uauy et al. (1990) observed that supplementation of 0.8% nucleotides promoted intestinal growth and maturation of young rats, with the increase in the villus height and crypt depth. Silva et al. (2009) evaluated the effect of yeast extract on the performance of broiler chickens and observed a better food conversion within the period from 1 to 21 days. Several studies have been carried out with another additive that deserves prominence in the scientific literature, the prebiotics (Albino et al., 2006; Oliveira et al. 2007, 2009; Godoi et al., 2008). It is known that the main form of action of the prebiotics is to favor the growth of benefic microbial populations, enhancing luminal conditions, anatomical characteristics of the gastrointestinal tract and immune system and also, in some cases, by enhancing the animal performance (Silva & Nörberg, 2003).

Environmental variables may have effect, such as positive or negative, on the production of broiler chickens. High temperatures cause metabolic changes in animals, according to reports in the literature, which results in lower meat yields, specially breast, lower muscle mass gain and higher fat accumulation, that impair performance.

Considering the importance of the research of new additives that may enhance beneficial effects in the gastrointestinal tract and, therefore, enhance the performance indexes and carcass yield in thermal stress situations, the objective of this study was to evaluate the effects of including yeast extract or prebiotic in the pre-initial phase on performance, carcass yield and intestinal morphometry of chickens raised under different temperatures.

Material and Methods

The experiment was conducted in the experimental poultry house of the Department of Animal Sciences of São Paulo State University, *Campus* Jaboticabal, with 1,440 male chicks of Cobb-500® lineage. The birds were raised in three climate chambers, consisting of 16 boxes, measuring 2.5 × 1.0 m each. The chambers were covered with polyurethane and were equipped with a heating and refrigeration system. The birds were submitted to different rearing temperatures (Table 1) with water and feed *ad libitum* throughout the experimental period of 42 days.

Table 1 - Environmental temperatures during the experimental period

Age (days)	Environmental temperature (°C)		
	Hot	Comfort	Cold
1 a 3	35 ± 1[1]	32 ± 2	28 ± 3
4 a 7	34 ± 1	31 ± 1	26 ± 2
8 a 14	32 ± 3	28 ± 2	22 ± 2
15 a 21	31 ± 3	26 ± 2	20 ± 3
22 a 42	30 ± 3	23 ± 2	19.5 ± 2

[1] Each value represents mean ± standard deviation of the mean.

The birds were vaccinated against Marek disease in the incubator house, against Gumboro (intermediate Lukert cepa) and Newcastle (La Sota estirpe) at 8 days and against Gumboro disease (Australia V-877 strong cepa) at 18 days. A light program of 24 hours was used.

Feed with or without the addition of yeast extract and prebiotic was offered only in the pre-initial phase (1 to 7 days). From the eighth day on, every bird received the same feed (Table 2), readjusted according to each rearing phase (1 to 7; 8 to 21 and 22 to 42 days), meeting the recommendations by Rostagno et al. (2000). Levels of yeast extract (2%) and prebiotic (0.15%) followed the recommendations of the manufacturer of the products.

A randomized complete experimental design was used in a 3 × 2 × 2 factorial arrangement, consisting of three rearing temperatures (hot, comfort, cold), levels of yeast extract (with or without) and prebiotic levels (with or without), in four replications of 30 birds per box in each climate chamber.

The chicken performance was evaluated by weight gain (WG), feed intake (FI), food conversion (FC) and broiler rearing viability (RV%) at 42 days of age. Feed intake was calculated from the difference between the weight of the feed offered and the leftovers in the troughs of the experimental units, also considering the mortalities in order to calculate each experimental unit.

At 42 days of age, four chickens from each replication were randomly selected, identified, numbered and submitted to 8 hours of fasting, weighed and processed, according to the normal procedures of stunning, slaughtering, plucking and evisceration. Carcasses were weighed with feet, neck and head, edible viscera and abdominal fat. Carcass yield was calculated based on live weight prior to slaughter and the yields of breast and whole leg (drumstick + thigh) were calculated based on carcass weight.

Two chickens from each replication were sacrificed at 42 days of age, after 12 hours fasting, to remove an empty gastrointestinal tract and to analyze the morphometric

Table 2 - Percentage, chemical and energetic composition of the experimental feed in the phases 1 to 7, 8 to 21 and 22 to 42 days of age

Ingredient	Phase					
	1 to 7 days				8 to 21 days	22 to 42 days
	1	2	3	4		
Corn	52.65	52.85	52.77	52.85	54.98	58.38
Soybean bran	39.19	37.03	39.16	37.03	36.63	31.14
Dicalcium phosphate	1.94	1.96	1.93	1.95	1.84	1.862
Limestone	0.93	0.87	0.87	0.87	0.87	0.83
Soybean oil	3.37	3.250	3.32	3.25	4.15	5.57
Salt	0.52	0.52	0.52	0.52	0.50	0.547
Mineral and vitamin supplementation	0.50[1]	0.50[1]	0.50[1]	0.50[1]	0.50[2]	0.50[3]
DL-methionine (99%)	0.39	0.42	0.38	0.41	0.27	0.21
L-lysine (78%)	0.37	0.45	0.37	0.45	0.21	0.23
Antioxidant	0.05	0.05	0.05	0.05	0.05	0.05
Inert material	-	0.10	-	-	-	-
Yeast extract[4]	-	2.00	-	2.00	-	-
Prebiotic[5]	-	-	0.15	0.15	-	-
Calculated composition						
Metabolizable energy (kcal/kg)	2,960	2,960	2,960	2,960	3,050	3,200
Gross protein (%)	21.11	21.14	21.11	21.11	21.14	19.20
Digestible lysine (%)	1.33	1.33	1.33	1.33	1.15	1.00
Digestible methionine (%)	0.52	0.52	0.52	0.52	0.45	0.41
Digestible methionine + cystine (%)	0.94	0.94	0.94	0.94	0.82	0.72
Calcium (%)	0.94	0.92	0.92	0.92	0.90	0.81
Available phosphorous (%)	0.47	0.47	0.47	0.47	0.45	0.40
Sodium (%)	0.21	0.21	0.21	0.21	0.19	0.19

[1] Mineral and vitamin suplementation. Composition/kg: Se, 54,6 mg; Cu, 25.000 mg; calcium pantothenate, 1.900; Mn, 15.252 mg; I, 260 mg; Zn, 18.250 mg; nicotinic acid, 6.930 mg; biotin, 32 mg; DL-methionine, 340 g; choline, 120 g; vit. A, 1.400.000 UI; vit. B1, 356 mg; vit. B12, 2.000 mcg; vit. B2, 1.920 mg; vit. B6, 693 mg; vit. D3, 600.000; vit. E, 5.000 mg; vit. K, 196 mg; antioxidant, 100 mg.
[2] = Mineral and vitamin suplementation [1]+ anticoccidial 25.000 mg and growth promoter 10.000 mg.
[3] from 35 to 42 days = Mineral and vitamin suplementation [1]+ growth promoter 10.000 mg.
[4] NuPro®: Alltech Agroindustrial from Brazil Ltda. Araucária, Paraná.
[5] Bio-Mos®: Alltech Agroindustrial from Brazil Ltda. Araucária, Paraná.

intestinal parameters. Samples approximately 2 cm long were removed from the medial region of each chicken, of the duodenum, jejunum and ileum of the small intestine. Fragments were opened longitudinally, removed and fixed immediately in *Bouin* solution for 24 hours. The samples were then washed in 70% alcohol to remove the fixing solution, dehydrated in increasing alcohol series, clarified in xylol and embedded in paraffin. Semi-seriated 5µm thick histological cuts were made, that were stained with hematoxilin-eosin, according to methodology by Behmer et al. (1976), and the microscope slides were assembled with Canada balsam. In order to asses the morphometric analyses of the histological microscope slides of the intestinal mucosa, the program *Image J*® (Rasband, 2004) was used to capture the images and to measure the villus height and crypt depth, with 64 readings/intestinal region/variable.

Intestinal samples were also collected from one of the chickens used for analysis in light field microscopy and for scanning electron microscopy study, a process performed in each replication. Fragments of approximately 2 cm of each segment had their intestinal content removed with phosphate buffer 0.01M and pH 7.4. Samples were fixed in 2.5% glutaraldehyde in phosphate buffer, for 24 hours at 4°C. After fixing, the fragments were washed again with the same buffer and post-fixed with osmium tetroxide at 1% in phosphate buffer for 2 hours. Samples were washed again in the same buffer solution and dehydrated in increasing alcohol series (30, 50, 70, 80, 90 and 100%). After achieving the critical point of dryness, using CO_2, the fragments were coated in gold and photographed in a scanning electron microscope (model: JEOL JSM 25SII®). The average number of villus/area/chicken was obtained by counting of the number of villi in three electron micrography/segment/chicken, where each area was equal to 117,673 μm^2.

Data was submitted to analysis of variance by the GLM procedure in the SAS® program (SAS, 2002) and, in case of significant difference, means were compared to determine the carcass and cuts yields and the morphometric analyses by Tukey test (5%) and for the other variables the Duncan or Fisher test (5%) were used.

Results and Discussion

There was significant interaction only between temperature and prebiotic in feed for weight gain (P=0.0273) and between temperature and yeast extract for food conversion (P=0.0267) (Tables 3 and 4, respectively). Prebiotic had significant effect only in the hot environment, a condition that provided greater weight gain at 42 days of age, when it was 5.33% greater than the weight gain obtained with feed without the inclusion of prebiotic (Table 3). This fact may be related to the higher villus height in the duodenum of chickens raised under this temperature that increased the absorption surface and provided a better use of the food (Figure 1).

When the effect of the prebiotic on temperature was evaluated, it was observed that when prebiotic was offered, weight gain was higher in the cold environment, followed by the comfort and hot environments, which did not differ statistically. The weight gain of chickens raised in this environment was on average 4.15% higher than that of chickens raised in the other temperatures. Greater weight gain in cold with the inclusion of prebiotic may be justified by the higher villus height in the duodenum and jejunum (Figure 1) and by a greater villus density in the jejunum, which provides a larger absorption area, increases weight gain, due to a better use of the food. However, in absence of prebiotic, weight gain was also higher in the cold environment, followed by the comfort and hot environments. In the cold environment, weight gain was 5.04% and 9.69% higher than in the chickens raised in the comfort and heat environments, respectively. Greater weight gain in the cold may be caused by the increase in the feed intake, as a mechanism to compensate the higher heat loss (Close & Mount, 1978). As counterpart, the lower weight gain in the hot environment happened due to less feed intake, as a defense mechanism to reduce heat production. Even if the animal produces heat by maintenance metabolism and possibly by the catabolism of some tissue, the heat generated by digestion and deposition in tissue is reduced. In addition, the villus height in the duodenum and jejunum of chickens fed diets without prebiotic was lower, despite the greater villus density found at this temperature.

Table 3 - Development of the interaction between environmental temperature and prebiotic for weight gain (g) of broiler chickens in the period from 1 to 42 days of age

Temperature	Prebiotic		Probability
	With	Without	
Hot	2,666Ab	2,524Bc	0.0020
Comfort	2,656b	2,654b	NS
Cold	2,771a	2,795a	NS
Probability	0.0145	<0.0001	

Means followed by the same lowercase (uppercase) letters at column (line) do not differ (P>0.05) according to the Fisher and Tukey tests.
NS = not significant.

Table 4 - Development of the interaction between environmental temperature and yeast extract for food conversion of broiler chickens in the period from 1 to 42 days of age

Temperature	Yeast extract		Probability
	With	Without	
Hot	1.58a	1.61a	NS
Comfort	1.71b	1.70b	NS
Cold	1.71Bb	1.65Aab	0.0436
Probability	<0.0001	0.0042	

Means followed by same lowercase (uppercase) letters in column (line) did not differ (P>0.05) according to the Fisher and Tukey tests
NS = not significant

Environmental temperature influenced food conversion (Table 4) at each level of yeast extract. The effect of cold temperature was more pronounced in the absence of yeast extract in the pre-initial feed, which provided better food conversion at the end of broiler raising. This fact may be related to greater villus height in the jejunum in the absence of yeast extract in the pre-initial feed (Figure 1).

When evaluating the inclusion of yeast extract at different temperatures, the worst food conversion was observed in the comfort and cold environments and the best was observed in the hot environment, but there were no difference between the results obtained in the cold and at other temperatures. The best food conversion in the hot environment occurred because of low food consumption and greater weight gain that resulted in better food conversion at that age. However, according to Abu-Dieyeh (2006), high temperature *per se* induces physiological changes in chickens with the decrease in metabolic rate, which results in less food intake and deficient digestion that impairs metabolism.

There was no interaction (P>0.05) of the results of feed intake, food conversion, rearing viability, carcass yield, breast percentage and whole leg percentage (Table 5).

Feed intake was lower in the hot environment compared to the comfort environment (P>0.0001), that, in turn, presented lower feed intake than in the cold environment (Table 5). Broiler chickens raised under hot temperatures consumed 9% less feed compared to those raised in the comfort environment. This occurred because, as feed intake increases the total heat production increases accordingly (Koh & Mcleod, 1999 a,b; Longo et al., 2006), and that increases the amount of heat received by the

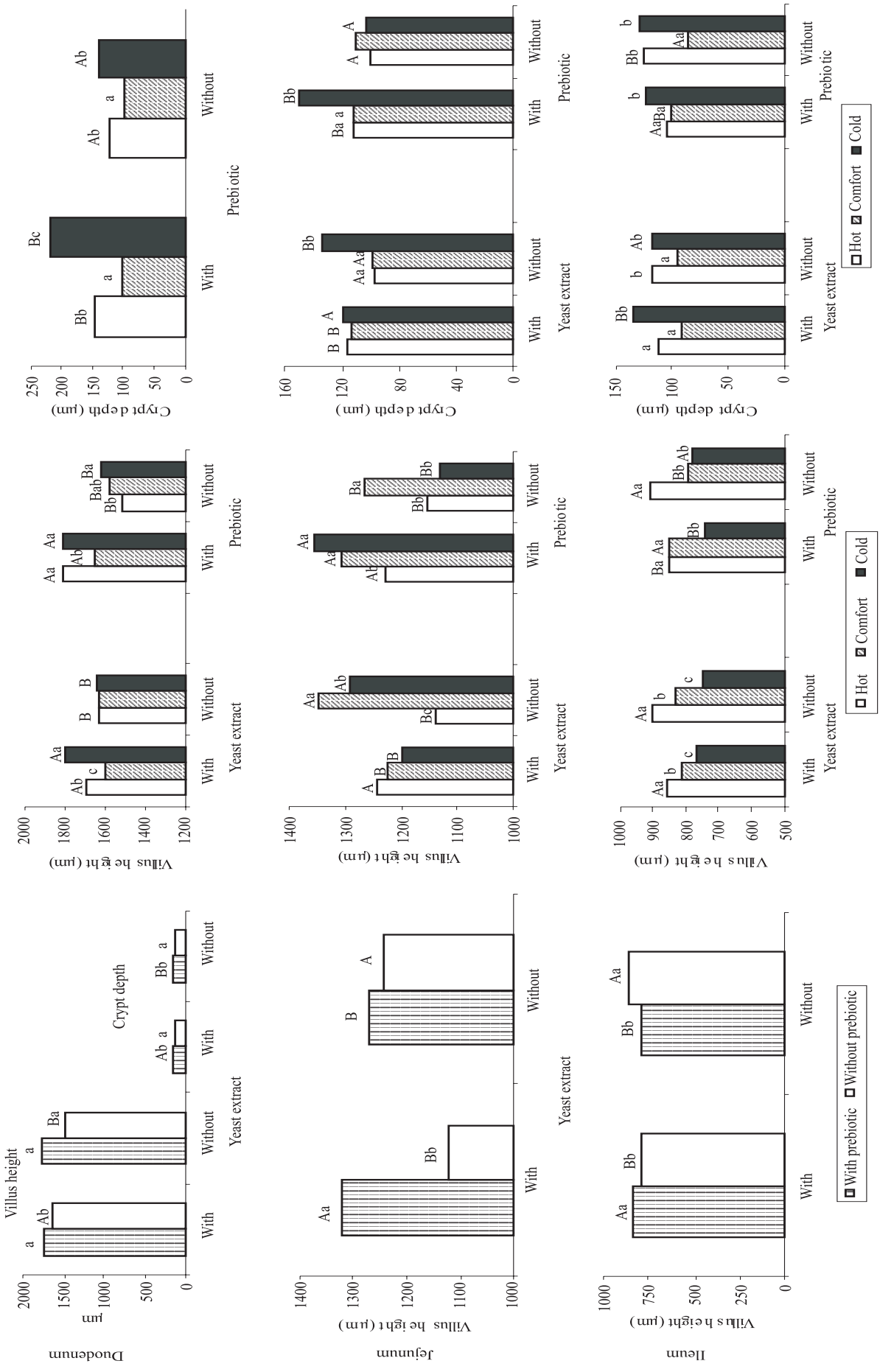

Figure 1 - Means of the interactions for villus height and crypt depth (µm) in the duodenum, jejunum and ileum at 42 days of age. A – Uppercase letters indicate effect of prebiotic at each yeast extract level and lowercase letters indicate effect of the yeast extract level at each level of prebiotic; B – Uppercase letters indicate effect of the prebiotic or yeast extract level in each environmental temperature and lowercase letters indicate the effect of the environmental temperature at each lprebiotic or yeast extract level.

Table 5 - Performance indexes of broiler chickens fed diets supplemented or not with yeast extract and prebiotic reared under different temperatures in the period from 1 to 42 days of age

	Feed intake (g)	Viability (%)	Carcass yield (%)	Breast (%)	Whole leg (drumstick + thigh) (%)
Temperature[1]					
Hot	4,134c	92.91	84.25a	31.42ab	27.58a
Comfort	4,507b	95.00	83.80b	31.60a	26.79b
Cold	4,676a	93.33	81.15b	30.93b	26.57b
Prebiotic[2]					
With	4,474a	95.41	83.00	31.47	27.04
Without	4,405b	92.08	83.13	31.29	26.92
Yeast extract[2]					
With	4,410	93.05	83.12	31.31	26.82
Without	4,468	94.44	83.01	31.45	27.14

[1] Means followed by the same letters in the same column do not indicate statistical differences (P>0.05) according to the Duncan test.
[2] Means followed by the same letters in the same column do not indicate statistical differences (P>0.05) according to the Fisher test.

animal, aggravating the caloric stress. When in a cold environment, chickens presented a 4% feed intake increase compared to the comfort environment, since when feed intake increases, heat production also increases, reducing the stress caused by cold.

None of the studied features affected the rearing viability in the 1 to 42 days of age phase (P>0.05). However, rearing viability was influenced by the inclusion of prebiotic in the diet of the phases 1 to 7 and 1 to 21 days of age. This beneficial effect may have been related to the small diversity of intestinal microflora of newborn chicks, because besides being considered a limiting factor for digestion, this also makes possible the intestinal colonization by enteric pathogens. The absence of contact with the natural microbiota prior to birth may affect the development of the gastrointestinal tract and impair the growth of chickens. Ito et al. (2004) described that, from the third day of the chick´s life, desirable microorganisms, such as *Lactobacillus* and others from the normal gastrointestinal microbiota, are found in large amounts in the intestinal medium. Nevertheless, the occurrence of greater challenges in environmental morbid situations may make the flora unstable until the 5th week of the bird life (Canalli et al., 1996). With the intestinal microbiota in equilibrium, birds had better conditions for absorbing nutrients and could confront, in better conditions, the stress in that production phase. Also, there was no influence of the yeast extract on weight gain, which is in agreement with results reported by Santos et al. (2005) who also did not observe the effect of prebiotics and organic acid on the performance of broiler chickens.

Only the environmental temperature affected the carcass yield (P=0.0003), breast (P=0.0469) and whole leg (P=0.0006) of the chickens. Carcass and whole leg yield swere greater in the hot than in the comfort and cold environments, with means 3.1% greater for carcass yield and 1% superior for whole leg yield (Table 5). The better carcass yield in the hot environment may have occurred because of the lower visceral development of birds reared at this temperature, due to the lower metabolism of the birds (Machado, 2001), the lower feather coverage, caused by the great need to dissipate heat (Geraert et al., 1996a; Cooper & Washburn, 1998), and to the greater deposition of abdominal fat (Howlider & Rose, 1987; Furlan et al., 2001). However, the higher carcass yield was not compensated by the higher body weight and by the better food conversion of birds reared in the hot environment.

Whole leg yield was greater in the birds (P=0.0006) in the hot environment, which may be explained by the oxidative metabolism in these muscles, because birds exposed to high temperatures require a higher concentration of plasmatic glucose that increases the oxidative metabolism and favors energy storage as fat in the whole leg (Geraert et al; 1996a,b; Faria Filho et al., 2006).

Breast yield was affected only by environmental temperature (P=0.0469) and was greater in the comfort environment, on average, 1% greater, and lower in the cold environment. Whereas in the hot environment the breast yield did not differ from those found in the comfort and cold environments. These results are in accordance with those reported by Perrault & Lesson (1992) and by Costa et al. (2001). However, they do not agree with the results presented by Baziz et al. (1996), Yalçin et al. (2001) and Faria Filho et al. (2006) where the lower breast yield in high temperatures was due to an increase in panting during heat stress that reflected in more activity of the pectoral girdle muscles, causing the breakdown of glycogen stored in these muscles, and thus impairing its yield. It is possible that the results found for breast yield may be related to the influence of hot and cold

environments since the first day of life, because in those experiments, birds were raised under high temperatures after 21 days of age. On the other hand, the effect caused by cold was inverse, because, as it is known, birds need high temperatures in their first days of life in order to maintain homeothermy but, in this experiment the stress caused by cold since the first day of life may be reflected within the first 42 days of life, resulting in lower breast yield.

Only the interactions between the environmental temperature and yeast extract for crypt depth in the duodenum, and between the prebiotic and yeast extract for crypt depth in the jejunum and ileum were not significant (Table 6).

The remaining effect of including prebiotic and yeast extract in the pre-initial feed was not enough to prevent the larger crypt depth found in the ileum at 42 days of age (P>0.05), probably because of an imbalance between two cellular renewal process – proliferation and differentiation (Figure 1), as a result of mitotic divisions of totipotent cells located in the crypt and along the villus (Uni et al., 1998).

It is known that, as the intestinal villi are destroyed, there is an attempt to repair the mucosa through the process of proliferation/mitosis in the crypt, determining deeper crypts (Blikslager & Roberts, 1997; Luquetti et al., 2006). According to Yason et al. (1987) and Pluske et al. (1997), higher values of crypt depth indicate more proliferative cell function to guarantee a suitable rate of epithelial renewal and high demand for new tissue. Nevertheless, data in the present study disagree from those reported by Bradley et al. (1994), who, when studying diet supplementation for broilers with 0.02% *S. cerevisae* observed a decrease in crypt depth in the ileum and suggested that the use of *S. cerevisae* reduced the stress conditions to which the mucosa is submitted and reduced the number of bacteria and toxins in the intestine.

Thermal stress caused by heat probably contributed to altering the release of thyroidal hormones that have enterotrophic action, as a stimulus for intestinal mucosa growth (Mitchell & Carlisle, 1992). Thus, functional hypothyroidism induced by caloric stress may be involved in the reduction of villus height in the duodenum (P>0.0001) and jejunum (P>0.0001) at 42 days. Few studies were found in the literature on the influence of temperature on intestinal morphometry.

Only the interactions between the environmental temperature and yeast extract for the duodenum and between prebiotic and yeast extract for the duodenum and ileum were not significant (P>0.05) (Table 7).

The influence of the temperature on villus density at 42 days of age was probably related to the stress level of the animal that also could influence the biological response obtained by adding prebiotics to the diet (Tables 8, 9 and 10). If the animals are kept in no-stress conditions, it is

Table 7 - Villus density of broiler chickens at 42 days of age

		Duodenum	Jejunum	Ileum
Environmental Temperature[1]	Hot	23.90[3]	32.89[3]	42.34b[3]
	Comfort	21.92	30.41	45.65b
	Cold	18.93	36.09	57.38a
Prebiotic[2]				
	With	21.09	33.48	50.65a
	Without	22.01	32.79	45.39b
Yeast extract[2]				
	With	25.45a	32.89	49.57a
	Without	14.83b	33.37	46.73b

[1] Means followed by the same letters in the same column do not indicate statistical differences (P>0.05) according to the Duncan test.
[2] Means followed by the same letters in the same column do not indicate statistical differences (P>0.05) according to the Fisher tests.
[3] Values expressed in number of villus/μm^2

Table 6 - Average values of villus height and crypt depth of broiler chickens fed diets supplemented or not with yeast extract and prebiotic and reared under different temperatures at 42 days of age

	Duodenum (μm)		Jejunum (μm)		Ileum (μm)	
	Villus height	Crypt depth	Villus height	Crypt depth	Villus height	Crypt depth
Temperature[1]						
Hot	1661b	134b	1192c	106b	879a	114b
Comfort	1614c	101c	1285a	106b	818b	93c
Cold	1719a	179a	1245b	127a	759c	126a
Prebiotic[2]						
With	1758a	134	1297a	124a	814	109
Without	1573b	142	1183b	102b	824	113
Yeast extract[2]						
With	1699a	156a	1222b	116a	812	112
Without	1631b	120b	1258a	110b	827	110

[1] Means followed by the same letters in the same column do not indicate statistical difference (P>0.05) according to the Duncan test.
[2] Means followed by the same letters in the same column do not indicate statistical difference (P>0.05) according to the Fisher test.

Table 8 - Development of the interaction between temperature and prebiotic for villus density in the duodenum of broiler chickens at 42 days of age

Temperature	Prebiotic		Probability
	With	Without	
Hot	21.17	26.64a	NS
Comfort	20.53	23.31a	NS
Cold	21.77	16.08b	NS
Probability	NS	0,0304	

In the same column, means followed by the same letters do not indicate statistical differences (P>0.05) according to the Tukey test.
Values expressed in number of villus/micrometer^2
NS = not significant

Table 9 - Development of the interaction for villus density in the jejunum of broiler chickens at 42 days of age

Prebiotic	Yeast extract		Probability
	With	Without	
With	37.30Aa	29.65Bb	0.0119
Without	28.47Bb	37.10Aa	0.0051
Probability	0.0042	0.0142	
Temperature	**Yeast extract**		**Probability**
	With	Without	
Hot	28.25Bb	37.54Aa	0.0126
Comfort	30.62b	30.18b	NS
Cold	39.79Aa	32.39Bab	0.0436
Probability	0.0024	0.0448	
Temperature	**Prebiotic**		**Probability**
	With	Without	
Hot	27.29Bb	38.50Aa	0.0031
Comfort	30.88B	29.92B	NS
Cold	42.25Aa	29.93Bb	0.0013
Probability	0.0002	0.0207	

Means followed by the same lowercase (uppercase) letters in column (line) do not differ (P>0.05) according to the Fisher and Tukey tests, respectively. NS = not significant
Values expressed in number of villus/µm^2

Table 10 - Development of the interactions for villus density in the ileum of broiler chickens at 42 days of age

Temperature	Prebiotic		Probability
	With	Without	
Hot	41.82b	41.88b	NS
Comfort	53.66Aa	37.64Bc	<0.0001
Cold	57.35a	57.42a	NS
Probability	<0.0001	<0.0001	
Temperature	**Yeast extract**		**Probability**
	With	Without	
Hot	39.44Bb	45.25Ab	0.0272
Comfort	54.77Aa	36.52 Bc	<0.0001
Cold	56.35a	58.41a	NS
Probability	<0.0001	<0.0001	

Means followed of same lowercase (uppercase) letters in column (line) do not differ according to the Fisher and Tukey tests (P>0.05).
NS = not significant
Values expressed in number of villus/µm^2

supposed that the microbiota would be in equilibrium, meaning that, with or without supplying prebiotics the answers obtained would be very similar. However, when the animals are submitted to a stressing condition, supplying with prebiotics also have a benefic effect upon the biological response (Mathew, 1993).

A possible explanation for the different villus densities in the ileum (Table 10) of birds reared under cold and hot temperatures at 42 days of age would be related to thyroidal hormones, that have an enterotrophic action by stimulating intestinal mucosa growth (Levin, 1969). Therefore, functional hypothyroidism induced by thermal stress could be involved in the decrease in the number of villi in that region (Mictchell & Carlisle, 1992). On the other hand, stress produced by cold enlarges the thyroid gland, because the functional activity of the thyroid, which is positively correlated to weight, is higher during the exposure to low temperature environments (Donkoh, 1989).

The beneficial action of the yeast extract on the villus density in the three intestinal regions was probably influenced by the thermal conditions and by the stress suffered by the animals (Table 9 and 10). When in a stress situation, alterations in the hypothalamo-hypophyseal-adrenal axis occur (Ballone, 2002), which reflect on hormone secretion and influence tissue development. Few data on the effect of yeast extract on villus density were found in the literature, but Uauy et al. (1990) and Ortega et al. (1995) suggested that the nucleotides may accelerate the normal physiological response to stress, but the mechanism is unknown.

Conclusions

Environmental temperature influences the performance and carcass yield that worsen in high temperature environments. The inclusion of prebiotic in pre-initial feed favors weight gain and enhances the food conversion in birds reared under high temperatures. Furthermore, it positively influences the morphometry and the villus density of the three intestinal regions evaluated; the duodenum and ileum are the regions that presents the best response under high and low temperature conditions and the jejunum is the region with the best response to the comfort temperature at 42 days of age.

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