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EVALUATION OF USING DIETARY PHYTOGENICS, AS GROWTH PROMOTERS, ON BROILER PERFORMANCE, UNDER NORMAL AND SUBNORMAL TEMPERATURE CONDITIONS

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SUMMARY

The present study was carried out to investigate the potential of dietary supplemental mixture of three phytogenics; carvacrol, cinnamaldehyde and capsicum oleoresin (Xtract$^{TM®}$) as growth promoter, under normal and subnormal temperature conditions. It was hypothesized that this mixture may alleviate the negative effects of cold stress on broilers performance and their immune response. Six hundred, one-day-old male broiler chicks, were subjected to one of two dietary treatments for 35 days: a basal diet as a control and the basal diet plus 100 ppm of Xtract$^{TM®}$. At 21days of age, half the birds in each dietary treatment were raised under one of two different ambient temperature treatments, the recommended normal temperature (22-24°C) versus a subnormal temperature (11-17°C), until 35 days of age. The results indicated that rearing broilers under subnormal temperature conditions, from 21 to 35 days of age, had significant negative effects on the production performance, carcass meat yield and immune response, as compared to those reared under normal temperature conditions. Under normal and subnormal temperature conditions, feed supplementation of 100 ppm Xtract$^{TM®}$ significantly increased final body weight, improved final feed conversion ratio, decreased total mortality rate, enhanced HI titers against Newcastle disease vaccine and consequently increased the profit potential of the birds, as compared to those not supplemented. Feed supplementation with Xtract$^{TM®}$ caused a significant increase in dressing percentage, breast meat, carcass meat as well as a significant decrease in abdominal fat. In addition, the Xtract$^{TM®}$ treated birds had significantly higher intestinal diameter and villus height than non treated ones. These results provide clear evidence to support the idea that the mixture of three phytogenics; carvacrol, cinnamaldehyde and capsicum oleoresin (Xtract$^{TM®}$) could be used as cost effective feed additive growth promoter in broilers up to 35 days of age, under normal as well as subnormal temperature conditions. Furthermore, Xtract$^{TM®}$ supplementation alleviated the negative effects of cold stress on production performance and immune response in broilers.

Keywords: broilers, phytoogenics, productive performance, immune response

INTRODUCTION

It has been well established that the usage of antibiotics, in animal diet, as growth promoters, is absolutely beneficial for the improvement of productive performance parameters and prevention of diseases (Karimi *et al*., 2010 and Puvaca *et al*., 2013). Nevertheless, because of the threats for human and animal health which come from resistant of pathogens to antibiotics and the accumulation of antibiotic residues in animal products and the environment (Stanacev *et al*., 2011), there is a global need to eliminate antibiotic growth promoters from animal diets. Earlier, antibiotic have been banned from animal and poultry diet by European Union since the year 2006. The demand for alternative products to antibiotics that can be used as prophylactic and growth promoting agents is very high. During the past several years, phytogenic compounds have attracted a lot of attention for their potential role as alternative to antibiotic growth promoters in animal nutrition (Puvaca *et al*., 2013).

The term "phytogenic compound" refers to the parts (*e.g.* seeds, fruits, roots, and leafs) of various aromatic herbs and spices (*e.g.* garlic, oregano, thyme, rosemary, coriander and cinnamon) as well as respective plant extracts in the form of essential oil (Windisch *et al*., 2008 and Stanacev *et al*., 2011). These compounds are also called phytonutrients or phytochemicals. Many beneficial properties of phytogenic compounds derive from their bioactive molecules (e.g. carvacrol, thymol, cineole, allicin, capsaicin and piperine) (Windisch *et al*., 2008).

The proposed mode of action of phytogenic compounds in poultry is attributed to their antimicrobial properties (Kollanoor-Johny *et al*., 2012, Renata *et al*., 2012 and Lee *et al*., 2013), antiviral (Burt, 2004), oxidative-resistant activity (Windisch *et al*., 2008), anti-inflammatory (Lee *et al*., 2009), enhancement of the immune system (Lillehoj *et al*., 2010 and Lee *et al*., 2013) and consequently, improvement in poultry performance. Besides efficacy, application of phytogenic feed additives to livestock also has to be safe to the animal, the consumer of the animal product, and the environment (Windisch *et al*., 2008).

Among these products, the mixture of three phytogenic compounds, namely carvacrol, cinnamaldehyde, and capsicum oleoresin, were reported as anti-bacterial and anti-fungal agents (Jamroz *et al*., 2005). Carvacrol is a component of numerous aromatic plants, such as Origanum vulgare, thyme, and wild bergamot (De Vincenzi *et al*., 2004). The anti-microbial functions of these plant extracts are associated with carvacrol, which inhibit *Salmonella* growth in chickens (Burt *et al*., 2005 and 2007). On the other hand, Lee *et al*. (2003) reported that dietary carvacrol lowered feed intake and weight gain but also lowered the feed-to-gain ratio. Cinnamaldehyde is a constituent of cinnamon and widely applied as flavoring. It has been proven to have strong anti-bacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus, Staphylococcus epidermidis,* and *Salmonella sp*. (Chang *et al*., 2001). Capsicum oleoresin, prepared by organic extraction of pepper fruits, contains anti-bacterial activity (Loizzo *et al*., 2008).

The results of the experiments that have been carried out to evaluate the response of broiler chicks to phytogenic compounds feed additives are inconclusive and not consistent and presented a scattered picture. Whereas some researchers have reported the full or partial effectiveness of phytogenic compounds in improving broiler performance (Jamroz *et al*., 2005, Burt *et al*., 2007 and Alali *et al*., 2013), others have reported no significant improvement (Hernandez *et al*., 2004, Lee *et al*., 2004a, Karimi *et al*., 2010 and Kollanoor-Johny *et al*., 2012). On the other hand, depressions in production performance due to phytogenic compounds feed additives were also reported (Cross *et al*., 2007). The broiler response to phytogenic compounds supplements is highly influenced by many factors such as environmental and sanitary conditions (Jamroz *et al*., 2005 and Hashemi and Davoodi, 2010).

Exposure of broiler chicks to temperatures, below the thermoneutral zone (18 to 24°C), after 3 weeks of age, has negative effects on the productive performance, meat yield, immune response, and mortality rate (Olanrewaju *et al*., 2010). Therefore, the present study was carried out to determine whether dietary supplemental mixture of carvacrol, cinnamaldehyde, and capsicum oleoresin (Xtract$^{TM®}$) could have the potential as growth promoter as well as alleviation of the negative effects of cold stress on broilers performance. The parameters evaluated were production performance, carcass yield, profit potential and immune response of broilers.

MATERIALS AND METHODS

Experimental design:

Six hundred one-day-old, feather-sexed male broiler chicks (Arbor Acres Plus) were obtained from a local hatchery, in December 2012 and were raised for 35days. They were randomly distributed to one of two open sided rooms (A&B). The rooms A and B had the same floor and windows areas and the same wind direction. Each room housed 300 chicks under two dietary treatments, each 150 chicks. Each treatment had 10 replicates of deep litter pens (1x1.5 m) with 15 birds per replicate. The two dietary treatments were as follows: control group= a basal diet and an Xtract$^{TM®}$ group= basal diet + Xtract$^{TM®}$ 6930, batch No. 57425 (produced by Pancosma, Switzerland), in a dosage according to the manufacturer’s recommendations (100 ppm). Xtract$^{TM®}$ 6930 is a mixture of three plant extracts containing 5.4% (wt/wt) carvacrol ($C_{10}H_{14}O$; from oregano, *Origanum spp*.), 3.2% (wt/wt) cinnamaldehyde (C_9H_8O, from cinnamon, *Cinnamonum spp*.) and 2.2% (wt/wt) capsicum oleoresin ($C_{18}H_{27}NO_3$ from Mexican pepper, *Capsicum annuum*) (Renata *et al*., 2012). This mixture was administered, in feed, via a premix on a vegetal carrier, from one-day-old until 35 days of age.

Ambient temperature treatments:

Standard brooding temperatures were maintained for all birds for the first 21 days of age. The temperature was set at 32^{o} C on the first day, gradually reduced to 24^{o} C at 21 days of age, when temperature treatments started. At 21 days of age, the birds were placed under two different temperature conditions treatments in rooms A and B. In room A, the birds were raised under the recommended normal temperature (NT group), 22 to 24°C, (Daneshyar *et al*., 2009) by using slight heating of the room. In room B, the birds were reared under subnormal temperature (SNT group), 11-17°C, by exposure to the ambient temperatures and winds in January, without heating, (January being the coldest month in Egypt's subtropical climate). Due to the cold climate, the minimum daily temperature recorded in room B varied from 10 to 12°C with a mean of 11°C. The maximum daily temperature ranged between 15 and 19°C with a mean of 17°C. In the two rooms, the temperature and humidity were recorded with thermohygrometers. The humidity ranged from 50 to 60%, from 21 to 35 days of age.

General management:

The composition of the diets and their calculated analysis are shown in Table 1. The commercial diets used were formulated to meet the nutrient requirements of the broiler chicks during starter, grower and finisher periods according to the National Research Council (NRC, 1994). Broilers were fed, *ad libitum*, a corn-soybean meal starter diet (23% crude protein and 3000 k cal ME/kg diet) during the first 2 weeks of age, a grower diet (21% crude protein and 3100 kcal ME/kg diet) from 2-4 weeks of age, and a finisher diet (19% crude protein and 3200 kcal ME/kg diet) during the 5th week of age. Starter feed was provided as crumbles, and subsequent feeds were provided as pellets. Semduramicin was added to the rations at a concentration of 25 ppm as a coccidiostat. Daily fluorescent illumination was applied for 23 h with 1 h of dark throughout the experimental period. Rooms were bedded with wood shavings and each replicate was equipped with 1 bell-shape drinker and 1 round tank feeder. All experimental birds were vaccinated against different diseases according to the vaccination programs adopted in most Egyptian chicken broiler farms (Elmenawey and Gharib, 2013). The vaccination program included vaccination against Newcastle disease (ND) using Hitchner B1 and La Sota strain live vaccines (via drinking water) at 7 and 18 days of age, respectively.

Table 1. Composition of the 3-phase diets (g/kg as fed) used and their calculated analysis

Ingredients	Starter (0-14 d)	Grower (15-28 d)	Finisher (29-35 d)
Yellow corn	524.5	544.2	628.5
Soybean meal 44%	332.4	299.1	221.1
Corn gluten meal 60%	70	70	66.5
Soya oil	30	43.8	40
Di-calcium phosphate	18	18	18
Lime stone	13	13	13
D.L. Methionine	2.2	2.1	2.3
Lysine hydrochloride	2.9	2.8	3.6
Sodium chloride	4	4	4
Premix*	3	3	3
Calculated analysis:			
Crude protein %	23.0	21.0	19.0
Metabolizable energy (kcal/kg)	3000	3100	3200

*Each gram of the premix contained: vitamin A (trans-retinyl acetate), 9,000 IU; vitamin D3 (cholecalciferol), 2,600 IU; vitamin E (dl-α-tocopheryl acetate), 16 mg; vitamin B1, 1.6 mg; vitamin B2, 6.5 mg; vitamin B6, 2.2 mg; vitamin B12 (cyanocobalamin), 0.015 mg; vitamin K3, 2.5mg; choline (choline chloride), 300 mg; nicotinic acid, 30 mg; pantothenic acid (d-calcium pantothenate), 10 mg; folic acid, 0.6 mg; d-biotin, 0.07 mg; manganese (MnO), 70 mg; zinc (ZnO), 60 mg; iron ($FeSO_4$ H_2O), 40 mg; copper ($CuSO_4$ $5H_2O$), 7 mg; iodine [$Ca(IO_3)_2$], 0.7 mg; selenium (Na_2SeO_3), 0.3 mg.

Measured parameters:

Production performance:

Individual body weights were obtained on d 1, 21, and 35 of age. Total feed consumption (FC), from 0-5 weeks of age in each replicate was calculated per bird (g/bird). The feed conversion ratio (FCR) was determined as the FC per body weight gain (g/g) for each replicate. Weight of the dead birds was used to adjust feed conversion. Mortality was recorded daily. Dead birds were subjected to postmortem examinations and those with accumulation of abdominal or pericardial fluids were diagnosed as ascites mortality (Ozkan *et al*., 2010). According to Timmerman *et al*. (2006), an index of productivity, so-called production number, which equals (Kilograms of growth per day * (100 - mortality %)) * 100 / Feed conversion ratio was calculated for each replicate, at the end of the experimental period.

Carcass yield:

At 35 days of age, 3 birds from each replicate were randomly chosen, fasted for 8 hours then weighed and slaughtered. After blood removal, they were defeathered, processed, and eviscerated. Determinations of carcass yield (dressing %, breast meat %, thigh with drumstick meat %, carcass meat %, abdominal fat %, and giblets (liver + heart + gizzard) %) were determined, as a percent of the fasting live body weights.

Intestinal length and diameter:

At 35 days of age, the small intestine of 3 birds, from each replicate (the segment between gizzard and ileocecal junction), was removed. It's length and diameter (in the middle of ileum) were measured.

Ileal mucosa:

At the end of the experiment, one bird from each replicate was chosen at random and sacrificed. Their small intestine was collected and immediately immersed in 10% buffered formalin. After fixation, 2 cm samples were taken from the middle of ileum. The ileum was considered from the Meckel's diverticulum to ileocecal junction (Samanya and Yamauchi, 2002). Routine histological laboratory methods were done (Zhang *et al*., 2005). The slides were stained with hematoxylineosin. Histological indices were measured using digital photography and light microscopy. The villus height (µm) was measured from the apical to the basal region, which corresponded to the superior portion of the crypts. Crypts depth (µm) was measured from the base until the region of transition between the crypt and the villus.

***Relative weights of spleen, thymus and bursa of Fabricius*:**

At 35 days of age, determination of the relative spleen, thymus and bursa of fabricius weights, as a percent of the fasting live body weights, were performed on 3 birds from each replicate.

Humoral Anti-ND vaccine antibody titers:

For determination of the effect of the temperature conditions and Xtract™® supplementation on humoral immunity; blood samples were collected from wing veins of 3 birds from each replicate, chosen at random, at 35 days of age. Serum samples were subjected to HI test for determining antibody titers against Newcastle disease (ND) vaccine as described by Swayne *et al*. (1998).

***Profit potential*:**

Profit potential was calculated as the income over cost, per each replicate, under normal or subnormal temperature conditions. The income was calculated as live body weight at 35 days of age, which was based on a price of 13 Egyptian pounds (LE) per kg. The cost included chick, feed and Xtract™® costs per chick. The chick price was LE 3.0; the mortality rate was used to adjust the chick cost. Feed cost was calculated from the actual feed cost incurred during the course of the experiment. The price for one ton ration was LE 4300 for starter diet, LE 4200 for grower diet and LE 4100 for finisher diet. The price for one kg of Xtract™® was LE 200.

Statistical analyses:

Two-way analysis of variance was done using the SAS GLM software procedure (SAS Institute, 1999). The main factors were temperature conditions and Xtract™® supplementation. Percentage data were subjected to arc-sine transformation prior to analysis. Mean values were compared using Duncan's Multiple Range Test (Duncan, 1955) when significant differences existed. Significance was set at $P<0.05$.

RESULTS

Production performance:

Results of the effects of temperature conditions and feed supplementation with mixture of carvacrol, cinnamaldehyde and capsicum oleoresin (Xtract™®) on production performance in broilers are shown in Table 2. Birds grown under subnormal temperature (SNT) conditions had significantly lower body weights, at 35 days of age, than those grown under normal temperature (NT) conditions. The results revealed that, in general, there were significant increases in final body weight, at 35 days of age, for the birds that received the Xtract™® (100 mg/kg diet) over the non treated group. The data of body weight, at 35 days of age, indicated a significant interaction between the temperature conditions and Xtract™® supplementation. Birds reared under NT and consumed diet with Xtract™®, had significantly the heaviest body weights. However, birds that were reared under SNT and consumed the basal diet had significantly the lowest body weights. The other two treatment groups (birds under NT with no Xtract™® and birds under SNT plus Xtract™®) body weights were intermediate with no significant differences between them.

Table 2. Effects of temperature conditions and feed supplementation with Xtract™® on production performance of broilers

Traits	Body weight (g)			Total FC[2] (g/bird)	FCR[3]	Total mortality %	Production number
Main effects	One day	21 days	35 days				
Temperature conditions (T) *							
Normal temperature (NT)	46.4	834.9	2120 ^{a}	3382	1.595 ^{b}	3.42 ^{b}	359.1 ^{a}
Subnormal temperature (SNT)	46.5	837.1	2046 ^{b}	3413	1.660 ^{a}	4.97 ^{a}	327.6 ^{b}
SEM[1]	0.2	8.7	14	34	0.011	0.34	7.5
Probability	NS	NS	<0.001	NS	<0.01	<0.001	<0.01
Xtract™® supplementation (X)							
No Xtract™® group (NXS)	46.5	834.5	2033 ^{b}	3378	1.658 ^{a}	4.57 ^{a}	327.0 ^{b}
Xtract™® group (XS)	46.4	837.6	2133 ^{a}	3417	1.597 ^{b}	3.82 ^{b}	359.7 ^{a}
SEM	0.3	8.4	15	35	0.012	0.23	7.8
Probability	NS	NS	<0.001	NS	<0.01	<0.01	<0.01
T * X Interaction							
NT * NXS	46.5	835.2	2064 ^{b}	3360	1.627 ^{b}	3.83 ^{c}	340.7 ^{b}
NT * XS	46.3	834.6	2175 ^{a}	3404	1.563 ^{c}	3.00 ^{d}	377.5 ^{a}
SNT * NXS	46.6	833.7	2001 ^{c}	3395	1.688 ^{a}	5.31 ^{a}	313.3 ^{c}
SNT * XS	46.5	840.5	2090 ^{b}	3430	1.631 ^{b}	4.63 ^{b}	341.9 ^{b}
SEM	0.2	7.6	14	33	0.010	0.25	7.1
Probability	NS	NS	<0.001	NS	<0.01	<0.001	<0.01

[a,d] Means, within a column and effect, followed by different superscripts differ significantly.
[*]Temperature treatment started at 21 days of age. [1]SEM = Stander error of the mean.
[2] FC = Feed consumption [3]FCR = FC (g) / weight gain (g)

Data of total feed consumption (Table 2) indicated that there were no significant effects due to either temperature conditions or Xtract™® supplementation, or the interaction between them. Rearing the birds under SNT conditions resulted in significantly higher final feed conversion ratio, as compared to NT conditions (Table 2). The addition of Xtract™® significantly improved the final feed conversion ratio. There was a significant interaction between the two treatments on the final feed conversion ratios. Birds that were reared under SNT conditions and consumed the basal diet had significantly the worst feed conversion. Whereas, birds reared under NT conditions and consumed the Xtract™® diet had the best feed conversion. The other two treatment groups were intermediate with no significant differences between them.

Regardless of the two treatments, no mortality due to ascites was observed (Table 2). Significantly higher mortality rate was present in the SNT group, compared with NT birds. The supplementation of Xtract$^{TM®}$ significantly decreased the total mortality percentage. Our data indicated a significant interaction between the temperature conditions and Xtract$^{TM®}$ supplementation on the total mortality percentage. Birds that were reared under SNT conditions and fed the basal diet had the highest total mortality percentage. However, the lowest total mortality percentage was observed in the NT plus Xtract$^{TM®}$ group. The other two experimental groups were intermediate, with the NT plus basal diet group had, significantly, lower total mortality than the SNT plus Xtract$^{TM®}$ group.

The SNT conditions resulted in a significant decrease in the production number as compared to NT conditions (Table 2). However, the Xtract$^{TM®}$ group had significantly higher production number than the not supplemented group. Significant interaction between the two treatments on the production number was also observed. Birds in the NT plus Xtract$^{TM®}$ group had the highest production number. However, the lowest production number was that of the SNT plus basal diet group. The other two treatment groups were intermediate with no significant differences between them.

Carcass yield:

Data of carcass yield, expressed as percentages of the live body weight, are presented in Table 3. Birds subjected to the SNT conditions had significantly lower breast and carcass meat yields, and abdominal fat deposition compared to the NT conditions group. There were no significant temperature conditions effects on dressing, thigh with drumstick meat or giblets percentages. The effect of feed supplementation with Xtract$^{TM®}$ on carcass yield resulted in significant increases in dressing %, and breast and carcass meat percentages as well as a significant decrease in abdominal fat %. There were significant interaction between the two treatments on dressing percentage, breast and carcass meat, and abdominal fat percentages. Under normal or subnormal temperature conditions, birds that consumed Xtract$^{TM®}$ feed had significantly higher percentages of dressing, breast meat and carcass meat, as compared to those received the basal diet. Birds in the NT plus Xtract$^{TM®}$ group had the lowest abdominal fat percentage, compared to the rest of the groups.

Table 3. Effects of temperature conditions and feed supplementation with Xtract$^{TM®}$ on carcass yield[1] of broilers, at 35 days of age

Traits / Main effects	Dressing %	Breast meat %	Thigh + drumstick meat %	Carcass meat %	Abdominal fat %	Giblets %
Temperature conditions (T)						
Normal temperature (NT)	69.08	20.12 ^{a}	15.39	35.50 ^{a}	1.32 ^{a}	4.60
Subnormal temperature (SNT)	69.00	18.72 ^{b}	15.41	34.13 ^{b}	1.11 ^{b}	4.69
SEM[2]	0.33	0.31	0.35	0.30	0.07	0.10
Probability	NS	<0.01	NS	<0.05	<0.01	NS
Xtract$^{TM®}$ supplementation (X)						
No Xtract$^{TM®}$ group (NXS)	68.15 ^{b}	18.76 ^{b}	15.28	34.04 ^{b}	1.31 ^{a}	4.65
Xtract$^{TM®}$ group (XS)	69.93 ^{a}	20.08 ^{a}	15.51	35.59 ^{a}	1.12 ^{b}	4.64
SEM	0.28	0.41	0.29	0.32	0.06	0.11
Probability	<0.05	<0.01	NS	<0.05	<0.01	NS
T * X Interaction						
NT * NXS	68.19 ^{b}	19.40 ^{b}	15.25	34.65 ^{b}	1.45 ^{a}	4.58
NT * XS	69.96 ^{a}	20.84 ^{a}	15.52	36.35 ^{a}	1.18 ^{b}	4.62
SNT * NXS	68.11 ^{b}	18.12 ^{c}	15.31	33.43 ^{c}	1.15 ^{b}	4.72
SNT * XS	69.89 ^{a}	19.31 ^{b}	15.49	34.80 ^{b}	1.06 ^{b}	4.66
SEM	0.28	0.39	0.25	0.35	0.07	0.08
Probability	<0.05	<0.05	NS	<0.05	<0.05	NS

[a,c] Means, within a column and effect, followed by different superscripts differ significantly.
[1] carcass yield expressed as percentages of the fasting live body weight.
[2] SEM = Stander error of the mean.

Intestinal length and diameter:

The results presented in Table 4 indicated that the temperature conditions had no significant effects on intestinal length or diameter. However, the Xtract$^{TM®}$ treated birds had significantly wider intestinal diameter, as compared to the not treated group. No significant differences were observed in the intestinal length due to the Xtract$^{TM®}$ supplementation.

There was significant interaction between temperature and Xtract$^{TM®}$ treatments on the intestinal diameter. Under normal or subnormal temperature conditions, birds consumed Xtract$^{TM®}$ feed had significantly wider intestinal diameter, as compared to the no Xtract$^{TM®}$ fed group. Within the Xtract$^{TM®}$ treatment, no significant temperature effects on the intestinal diameter were observed.

Table 4. Effects of temperature conditions and feed supplementation with Xtract$^{TM®}$ on intestinal morphology and ileal mucosa morphology of broilers, at 35 days of age

Traits	Intestinal morphology		Ileal mucosa morphology		
Main effects	Intestine length (cm)	Intestine diameter (cm)	Villus height (µm)	Crypt depth (µm)	Villus height / Crypt depth ratio
Temperature conditions (T)					
Normal temperature (NT)	188.9	0.982	362	118	3.09
Subnormal temperature (SNT)	183.0	0.977	344	123	2.82
SEM[1]	7.2	0.092	22	12	0.25
Probability	NS	NS	NS	NS	NS
Xtract$^{TM®}$ supplementation (X)					
No Xtract$^{TM®}$ group (NXS)	182.8	0.897 ^{b}	312 ^{b}	124	2.52 ^{b}
Xtract$^{TM®}$ group (XS)	189.1	1.061 ^{a}	395 ^{a}	117	3.38 ^{a}
SEM	8.1	0.046	26	15	0.23
Probability	NS	<0.001	<0.01	NS	<0.01
T * X Interaction					
NT * NXS	185.8	0.900 ^{b}	322 ^{b}	122	2.64 ^{b}
NT * XS	191.9	1.063 ^{a}	402 ^{a}	114	3.53 ^{a}
SNT * NXS	179.7	0.894 ^{b}	301 ^{b}	125	2.41 ^{b}
SNT * XS	186.2	1.059 ^{a}	387 ^{a}	120	3.23 ^{a}
SEM	7.9	0.031	21	14	0.21
Probability	NS	<0.01	<0.05	NS	<0.01

[a,c] Means, within a column and effect, followed by different superscripts differ significantly.
[1]SEM = Stander error of the mean.

Ileal mucosa:

The effects of temperature conditions and feed supplementation with Xtract$^{TM®}$ on the ileal mucosa, of 35-day old male broiler chickens are presented in Table 4. There were no significant effects, due to temperature conditions, on the ileal mucosa morphology. Whereas, birds fed Xtract$^{TM®}$ diet had significantly greater villus height and villus height/crypt depth ratio, as compared to the not treated group. However, no significant differences were found in crypt depth due to the Xtract$^{TM®}$ supplementation. Significant interaction effects between the two treatments on villus height and villus height/crypt depth ratio were also observed. Within the temperature conditions, birds that consumed the Xtract$^{TM®}$ diet had significantly higher villus height and villus height/crypt depth ratio, as compared to the no Xtract$^{TM®}$ diet group. Within the Xtract$^{TM®}$ treatment, no significant effects due to the temperature conditions were observed on the villus height or villus height/crypt depth ratio.

Relative weights of spleen, thymus and bursa of Fabricius:

There were no significant effects of either temperature conditions or Xtract$^{TM®}$ supplementation or the interaction between them on the relative weights of spleen, thymus or bursa of Fabricius, for 35-day old male broilers (Table 5).

Humoral Anti-ND vaccine antibody titers:

Serum antibody responses to vaccination against ND antigen that were determined at 35 days of age are presented in Table 5. Growing birds under SNT conditions resulted in significantly lower antibody titers against ND vaccine. The HI titers against ND vaccine were significantly higher in the Xtract$^{TM®}$

group as compared to the no Xtract$^{TM®}$ group. There was a significant interaction of the two treatments on the HI titers against ND vaccine. Birds in NT plus Xtract$^{TM®}$ group had significantly higher HI titers against ND vaccine, as compared to birds in NT plus basal diet and SNT plus basal diet groups. However, the differences between the SNT plus Xtract$^{TM®}$ and NT plus basal diet groups were not significant.

Profit potential:

Table (6) summarizes the profit potential of Xtract$^{TM®}$ supplementation, under normal or subnormal temperature conditions. It was noted that feed supplementation with Xtract$^{TM®}$ resulted in significant increase in profit potential, as compared to the not supplemented groups, under normal or subnormal temperature conditions. The profit potential was higher in the Xtract$^{TM®}$ fed broilers than the not Xtract$^{TM®}$ ones by about LE 1.22 and 0.98 per bird, under the normal or subnormal temperature conditions, respectively.

Table 5. Effects of temperature conditions and feed supplementation with Xtract$^{TM®}$ on relative weights of lymphoid organs and HI titers against Newcastle disease (ND) vaccine in broilers, at 35 days of age

Traits / Main effects	Relative weights of lymphoid organs			HI titer against ND vaccine
	Spleen	Thymus	Bursa of Fabricius	
Temperature conditions (T)				
Normal temperature (NT)	0.230	0.717	0.264	6.64^{a}
Subnormal temperature (SNT)	0.288	0.714	0.261	5.71^{b}
SEM[1]	0.04	0.06	0.02	0.23
Probability	NS	NS	NS	<0.01
Xtract$^{TM®}$ supplementation (X)				
No Xtract$^{TM®}$ group (NXS)	0.226	0.709	0.265	5.56^{b}
Xtract$^{TM®}$ group (XS)	0.232	0.723	0.260	6.80^{a}
SEM	0.05	0.08	0.03	0.25
Probability	NS	NS	NS	<0.01
T * X Interaction				
NT * NXS	0.227	0.700	0.267	6.10^{b}
NT * XS	0.233	0.727	0.260	7.18^{a}
SNT * NXS	0.225	0.709	0.262	5.01^{c}
SNT * XS	0.231	0.718	0.260	6.41^{ab}
SEM	0.04	0.09	0.03	0.35
Probability	NS	NS	NS	<0.01

[a,c]Means within a column and effect, followed by different superscripts differ significantly ($P \le 0.05$).
[1]SEM = Stander error of the mean.

Table 6. Effects of feed supplementation with Xtract$^{TM®}$ on profit potential per bird (Egyptian pound), of broilers, under normal or subnormal temperature conditions

Item (Egyptian pound/ bird)	Under normal temperature Conditions		Under subnormal temperature conditions	
	No Xtract$^{TM®}$	Xtract$^{TM®}$	No Xtract$^{TM®}$	Xtract$^{TM®}$
Chick cost[1]	3.11 ± 0.05[*]	3.09 ± 0.06	3.16 ± 0.06	3.14 ± 0.06
Feed cost	14.01 ± 0.15	14.19 ± 0.18	14.16 ± 0.17	14.30 ± 0.19
Xtract$^{TM®}$ cost	0.00	0.07	0.00	0.07
Total cost	17.12 ± 0.19	17.35 ± 0.21	17.32 ± 0.22	17.51 ± 0.25
Income	26.83 ± 0.24^{b}	28.28 ± 0.26^{a}	26.01± 0.23^{B}	27.18 ± 0.22^{A}
Profit potential	9.71 ± 0.12^{b}	10.93 ± 0.17^{a}	8.69 ± 0.11^{B}	9.67 ± 0.14^{A}

[a,b] Means, within item, within normal temperature conditions, between Xtract$^{TM®}$ treatments, followed by different superscripts differ significantly ($P \le 0.05$).
[A,B] Means, within item, within subnormal temperature conditions, between Xtract$^{TM®}$ treatments, followed by different superscripts differ significantly ($P \le 0.05$).
[*]Means ± Stander error. [1] reflects mortality.

DISCUSSION

The present results indicate that rearing broilers under subnormal temperature conditions, from 21 to 35 days of age, had significant negative effects on their production performance parameters and their immune response, as compared to those reared under the recommended normal temperature. These results are in agreement with those reported by Olanrewaju *et al*. (2010), who found that exposure of broiler chicks to temperatures below the thermoneutral zone (18 to 24°C), at 3 weeks of age; adversely affect productive performance, meat yield, immune response, and mortality rate. Furthermore, they stated that, in broilers, even relatively small changes in the temperature conditions from the thermoneutral zone can have a negative effect on their metabolism and performance. Birds subjected to cold conditions had significantly lower carcass yield, poorer feed conversion (Akşit *et al*., 2013) and higher mortality rate (Ozkan *et al*., 2010) compared to those reared under recommended normal temperature conditions.

The observed significant reduction in the production performance and immune response, of the broilers, due to the low temperature condition, may be due to an increase in heat production, which increases the oxygen requirement of the body (Ozkan *et al*. 2010). It was also reported that low oxygen availability might act as a growth-restricting factor and increase the mortality in birds (Daneshyar *et al*., 2009). It was reported that poor heart performance may be the main cause for bird's death, which leads to significant economic losses under cold conditions (Druyan *et al*., 2007). Poor heart performance is a major factor in the pathogenesis of hypoxemia in fast-growing broilers (Olkowski, 2007). It is also known that nutrient digestibility decrease when ambient temperature goes below the thermoneutral zone, and the food energy will be directed towards maintenance to sustain normal biological activities rather than towards growth (Sahin and Sahin, 2002).

Prediction of the broiler response to phytogenic compounds supplements is not clear because it is highly influenced by many factors such as the environmental conditions (Jamroz *et al*., 2005 and Hashemi and Davoodi, 2010).The current results indicate that feeding Xtract$^{TM®}$ (a mixture containing 5.4% carvacrol, 3.2% cinnamaldehyde, and 2.2% capsicum oleoresin) to broilers from 1- 35 days of age, had significant improvement effects on production performance, intestinal histomorphological parameters, immune response and a significant profit, under normal and subnormal temperature conditions. Moreover, the Xtract$^{TM®}$ supplementation alleviated the negative effects of cold stress on production performance and immune response.

The present results are in agreement with those reported by Jamroz *et al*. (2005) and Bravo *et al*. (2011). They reported that, the addition of 100 mg/kg of the Xtract$^{TM®}$ in the broiler diet increased broiler growth performance and improved the feed conversion ratio. Alali *et al*. (2013) reported that an essential oil blend (EO; carvacrol, thymol, eucalyptol, lemon) administered in the drinking water of broiler chickens resulted in significant improvements in feed conversion ratio and body weight gain.

On the other hand, our results disagree with those reported by Alp *et al*. (2012). They found that supplementing diets with oregano essential oil had no significant effects on the carcass yield, or serum IgG level, in broiler chickens at 42 days of age. It was also reported that the essential oil components (carvacrol and cinnamaldehyde) did not affect small intestinal length (Lee *et al*., 2004b), carcass yield or abdominal fat percentages, but increased weight of jejunum (Tekeli *et al*., 2006). The differences between these results and our results may be due to the differences in the essential oil components, dose and duration of the supplementation, age of the birds, environmental conditions or the hygienic conditions (Jamroz *et al*., 2005, Windisch *et al*., 2008 and Hashemi and Davoodi, 2010).

The positive effects of Xtract$^{TM®}$ supplementation on the production performance could be related to the improvement of intestinal diameter and villi height that allows larger surface available for nutrient absorption. Potential effects of phytogenic additives on gut morphological characteristics have been reported by Jamroz *et al*. (2006). Hernandez *et al*. (2004) demonstrated that Xtract$^{TM®}$ significantly improved apparent ileal digestibility coefficients for nutrients in broilers diets. Carvacrol and cinnamaldehyde have been shown to increase the activity of the antioxidant enzymes of the cells of the mucosa layer (Dhuley, 1999), which is known to be a protective system for the tissue. The latter could be the basis for the mucosal protective effect previously observed with Xtract$^{TM®}$ (Jamroz *et al*., 2006), who found a thicker layer of mucus on the wall of the glandular jejunum, suggesting a villus-related protective effect of Xtract$^{TM®}$. This may explain the positive effects of Xtract$^{TM®}$ on the intestinal diameter and ileal mucosa (Mitsch *et al*., 2004 and Cross *et al*., 2007) in the present study.

Consequently, dietary factors that improve the morphology of the small intestinal mucosa may improve energy utilization. Bravo *et al*. (2011) confirmed that the addition of 100 mg/kg of Xtract$^{TM®}$ in the broiler diet increased broiler growth performance and improved energy utilization by, at least, 50 kcal/ kg. Thus, a diet containing 2,950 kcal of ME/kg plus Xtract$^{TM®}$ resulted in performance that was equivalent to that of a 3,000 kcal of ME/kg diet without Xtract$^{TM®}$. They explained that the effect of Xtract$^{TM®}$ may be mediated through a direct increase in dietary energy digestibility or absorption, by a decrease in the energy required for the maintenance of the digestive tract, or by a combination of both. These observations give further support to the hypothesis that the mixture of these three phytogenics feed additives may stabilize digestive functions and could alleviate the negative effects of cold stress on broilers performance.

The reason for the enhanced digestibility with Xtract$^{TM®}$ could also be due to increased digestive secretions, as reported for the effect of capsicum oleoresin on pancreatic and intestinal enzyme activity, bile flow and bile acid secretion (Jamroz *et al*., 2005). Additionally, recent application of high-throughput genomics technology has demonstrated that carvacrol, cinnamaldehyde, and capsicum oleoresin are able to alter the expression of 74, 62, and 254 genes involved in metabolism (Kim *et al*., 2010).

Furthermore, the observed positive effects of Xtract$^{TM®}$ on the production performance and immune response can be attributed to the essential oils present in this mixture (carvacrol, cinnamaldehyde, and capsicum oleoresin) and its active constituents which possess antibacterial, antifungal and antioxidant activities due to the presence of phenolic compounds (Lee *et al*., 2004a, Kollanoor-Johny *et al*., 2012 and Renata *et al*., 2012). Carvacrol has been shown to inhibit *Salmonella* growth (Burt et al., 2007) and *Escherichia .coli* (Renata et al., 2012), in chickens. Cinnamaldehyde has been proven to have strong anti-bacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Salmonella sp.* (Chang *et al*., 2001).

Furthermore, Kollanoor-Johny *et al*. (2012) reported that Carvacrol and Cinnamaldehyde exhibit a wide range of antimicrobial activity and act synergistically when combined. They suggested that a blend of essential components may be more effective in improving growth performance than an identical amount of an individual component alone. Also, capsicum oleoresin contains anti-bacterial activity (Loizzo *et al*., 2008). Moreover, Renata *et al.* (2012) showed that mixture of carvacrol, cinnamaldehyde, and capsicum oleoresin administration caused a reduction in *Eimeria sp.* oocysts. Therefore, the lower mortality rate in Xtract$^{TM®}$ group may also be attributed to the growth promotion action against pathogenic micro-organisms that can attack young chicks.

In addition, Lillehoj *et al*. (2010) reported that analysis of global gene expression profiles of intestinal tissues from phytgenics-fed birds indicated that capsicum oleoresin induced the most gene changes compared to the control group, where many of these genes were associated with those of immunity. The most reliable network induced by dietary cinnamaldehyde treatment was related to the functions of antigen presentation, humoral immune response, and inflammatory disease. They stated that the phytogenics carvacrol, cinnamaldehyde, and capsicum oleoresin have shown beneficial effects on host immune system through the regulation of gene expression in the chicken gut. These may explain the evidence of positive effects of Xtract$^{TM®}$ on the immune response and mortality rate in the present study.

In summary, the results of the present study indicate that, the mixture containing phytogenics; carvacrol, cinnamaldehyde, and capsicum oleoresin (Xtract$^{TM®}$) could be used as economical growth promoter in broilers, via feed, under normal and subnormal temperature conditions. Additionally, it could alleviate the negative effects of cold stress on broilers production performance and enhance their immune response.

CONCLUSIONS

In conclusion, a commercial mixture (Xtract$^{TM®}$) containing three phytogenics (carvacrol, cinnamaldehyde, and capsicum oleoresin) was proved to increase final body weight, improve final feed conversion ratio, decrease total mortality percentage, enhance HI titers against ND vaccine and increase profit potential, in broilers. These results provide clear evidence to support the idea that the phytoogenics; carvacrol, cinnamaldehyde, and capsicum oleoresin could be used as economical feed additive growth promoter in broilers, up to 35 days of age. Moreover, Xtract$^{TM®}$ supplementation may alleviate the negative effects of cold stress on broilers production performance and immune response.

Further research on the effect of these phytoogenics on broilers performance and immune response under other stressful conditions should be done. With respect to consumer safety, further research is still needed to study the effects of this mixture on the broilers meat quality.

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تقييم استخدام مركبات الفيتوجينك على العليقة كمنشط للنمو لكتاكيت انتاج اللحم المرباه تحت ظروف درجات الحرارة العادية أو المنخفضة

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تم إجراء هذه الدراسة لتقييم إضافة خليط يشتمل على ثلاثة من مركبات الفيتوجينك (كرفكرول – سيناملدهيد – مستخلص الفلفل الحار) تحت الاسم تجاري (Xtract™®) لعليقة دجاج انتاج اللحم ليعمل كمنشط للنمو في ظروف التربية تحت درجات الحرارة العادية أو المنخفضة. كما تم اختبار امكانية استخدام هذا المخلوط لتقليل التأثير السلبى للتربية تحت درجات الحرارة المنخفضة على الأداء الأنتاجى لدجاج انتاج اللحم.

تم تقسيم عدد 600 كتكوت أربور ايكرز بلس ذكر عمر يوم من كتاكيت إنتاج اللحم إلى معاملتين للعليقة حتى نهاية التجربة عند عمر 35 يوم: المعاملة الأولى كانت عليقة اساسية للمقارنة أما المعاملة الثانية كانت عليقة المقارنة مضاف إليها 100مجم من خليط Xtract™® لكل كجم من العليقة. عند عمر 21 يوم تم تعريض نصف عدد الكتاكيت في كل معاملة من معاملات العليقة لأحد معاملتين لدرجة حرارة العنبر حتى عمر 35 يوم: درجة حرارة عادية (22- 24°م) أو درجة حرارة منخفضة (11- 17°م).

أشارت النتائج إلى أن تربية كتاكيت إنتاج اللحم تحت ظروف درجة الحرارة المنخفضة من عمر 21 حتى 35 يوم أدي إلى إنخفاض الكفاءة الإنتاجية و محصول اللحم والاستجابة المناعية مقارنة بالدجاج المربى تحت ظروف درجة الحرارة العادية. كما اوضحت النتائج بأنه تحت ظروف درجات الحرارة العادية أوالمنخفضة وجد أن اضافة مخلوط Xtract™® الى العليقة أدى إلى زيادة معنوية فى وزن جسم الدجاج عند عمر 35 يوم ، تحسن معنوي في كفاءة التحويل الغذائي ، انخفاض معنوي فى نسبة النفوق الكلية وارتفاع معنوي فى مستوى الأجسام المناعية ضد لقاح النيوكاسيل وكذلك زيادة معنوية فى العائد الأقتصادي مقارنة بالدجاج المغذى على عليقة بدون Xtract™®. كما أدى الي زيادة معنوية في نسبة التصافى ونسبة لحم كل من الصدر والذبيحة وكذلك انخفاض في نسبة دهن البطن ولوحظ أن اضافة Xtract™® على العليقة أدى إلى زيادة قطر الأمعاء وأرتفاع الخملات.

هذه النتائج تؤيد النظرية بأن الخليط من مركبات الفيتوجينك (كرفكرول – سيناملدهيد – مستخلص الفلفل الحار) Xtract™® يعمل كمنشط نمو لدجاج إنتاج اللحم حتى عمر 35 يوم تحت ظروف درجات الحرارة العادية أوالمنخفضة , كما انه يقلل من التأثير السلبي لتربية دجاج انتاج اللحم تحت ظروف درجة الحرارة المنخفضة خلال الاسبوع الرابع والخامس من عمرها .