

ANTIOXIDATIVE STATUS AND MEAT SENSORY QUALITY OF BROILER CHICKEN FED WITH XTRACT® AND ZEOLITE DIETARY SUPPLEMENTATION

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The ban on use of antibiotic growth promoter for fattening chickens has created demand for other weight and health promoting supplements. Phytochemicals, and especially essential oils (EO) or their components, have attracted great interest. They are considered 'natural' and leaving no unwanted tissue residue. The aim of this study was to evaluate the effect of XTRACT® (carvacrol, cinnamaldehyde and capsicum oleoresin) and natural zeolite clinoptilolite as feed additives in broiler chicken. Addition of XTRACT® and/or zeolite had positive influence on antioxidative enzymes SOD and GPx during chicken fattening. This indirectly resulted in higher final body weight of chicken. Lower lipid peroxidation in breast meat and drumsticks was also observed in experimental groups. The sensory quality of chicken breast meat and drumstick meat, expressed as color, structure, juiciness, tenderness, odor and taste acceptability was improved in experimental groups.

Keywords: Chicken, meat, essential oils, zeolite, antioxidants.

INTRODUCTION

The ban of using antimicrobial growth promoters for broiler fattening created a research gap for commercially and socially acceptable replacement growth promoters such as plant derived biologically active compounds or phytochemicals. Compared to synthetically derived antibiotics and inorganic chemical substances, phytochemicals are often derived from plants, do not leave tissue residues and are proven less toxic (Hashemi *et al.*, 2008). Phytochemicals therefore have potential to become ideal feed additives if they can successfully replicate some of the effects of antibiotic growth promoters in feed. Phytochemicals include a wide range of herbs, spices and their derivatives, which are well known as essential oils (Perić *et al.*, 2009). The active compounds contained in phytochemicals are secondary plant metabolites called phytochemicals, some of which exhibit substantive antimicrobial properties, hepatoprotective, antimicrobial, antioxidant, antifungal effects while recent research showed also antiparasitic influence (Drăgan *et al.*, 2014). It requires further work to achieve the right combination of phytochemicals and dosages, Hashemi and Davoodi, (2010).

The positive effect of phytochemicals on chicken growth and health is mainly a consequence of their antimicrobial properties in conjunction with the ability to stimulate immunity (Yang *et al.*, 2009). Essential oils have antioxidant impact due to phenol component which contains a hydroxyl group, Shahidi and Wanasundara (1992). Oxidative stress can lead to biological cell damage and pathological conditions that can

influence chick's growth, Fellenberg and Speisky, (2006). This results in higher lipid peroxidation and production of reactive oxygen species (ROS) in serum and tissues, which in higher concentrations can damage cell function. Tissue damage can be effectively protected with enzymatic and non-enzymatic antioxidants which include superoxide dismutase (SOD) and glutathione peroxidase (GPx). In this sense SOD and GPx activity can be used as indicator for oxidative stress (Maini *et al.*, 2007). Antioxidants added to feed are absorbed in intestines and show systematic impact on these two markers, Fellenberg and Speisky, (2006). Polat *et al.* (2011) confirmed that polyphenols rich EO can raise SOD activity in serum, but only up until a certain dosage. Essential oils also have the ability to activate antioxidant enzymes, especially GPx, and further influence antioxidant protection (Faix *et al.*, 2009).

Another marker of oxidative stress is provided by the levels of malondialdehyde (MDA) in tissue. Free radicals from unsaturated free fatty acid, that aren't eliminated by cell itself, can easily oxidize leading to higher MDA concentration. Yasar *et al.* (2011) examined different herbs as chicken feed additives but did not observe changes in the MDA levels before and after free radical attack, or on GPx enzymatic activity. However, a statistically higher SOD activity was observed.

Other feed additives derived from mineral sources have also been investigated for their benefit effect on chicken farming. These include natural zeolites-clinoptilolites, with their crystalline three-dimensional structure, that could serve as

useful feed additive across chicken production with strong adsorptive and ion exchange capacity. These mineral additives have been shown to prevent mycotoxicosis (Šperanda *et al.*, 2006) and increase growth performance in broiler chicken (Karamanlis *et al.*, 2008).

The aim of this study was to evaluate the effect of XTRACT® (consist of three EO components: carvacrol, cinnamaldehyde and capsicum oleoresin) and natural zeolite clinoptilolite as feed additives in broiler chicken, both separately and in combination. Influence is evaluated in terms of on antioxidative values of SOD and GPx enzymes in blood, influence on broiler growth, cuts weights, lipid peroxidation in breast meat and drumstick, and meat sensory values.

MATERIALS AND METHODS

Experiment design. In this experiment, 192 one day old, Ross 308, mixed sex chicks were randomly allocated to 4 feed groups, in 3 replicates for each group with 16 chicks per replicate. The feeding program consisted of starter diet until day 15th, grower diet until day 25th and finisher diet until day 42nd (end of fattening).

The diet. The same diet was used for all groups and categories (starter, grower, and finisher) including the control (C) for the entire grow out period from day 1 to day 42 (Table 1). As a feed additive a standardized mixture of plant extracts (XTRACT® 6930; Pancosma S.A., Geneva, Switzerland), combined of 5% carvacrol, 3% cinnamaldehyde and 2% capsicum oleoresin, was used in the diet formulation in the 1st and 3rd experimental groups. The first experimental group (XT) received feed with 0.01% XTRACT®; the second experimental group (ZEO) received feed with 0.2% zeolite and third group (XT+ZEO) received feed with 0.01% XTRACT® and 0.2% zeolite. The zeolite clinoptilolite with a long chain quaternary ammonium salt was compound from the following components: SiO₂ 63-68%, Al₂O₃ 11-14%, Fe₂O₃ 0.8-2.5%, MnO 0.01-0.03%, CaO 2.5-4.5%, MgO 0.8-1.5%, Na₂O 0.8-1.5%, K₂O 1.0-2.0, L.I. 10.5-14.5%.

Table 1

Feed conversion ratio. Remaining feed was weighed on day 15 (starter), day 25 (grower) and day 42 (finisher) and difference used to calculate feed conversions ratio as per:

$$\text{Feed conversion ratio} = \frac{\text{total feed (kg)}}{\text{total weight (kg)}}$$

Broilers were weighed on day 42 both as live weight before slaughter and as dressed carcasses to determine saleable yield. Carcasses were further divided into wings, back, drumstick and breast and weighed separately for each group.

Carcass resection. Broiler carcass resection is done according to following principle: wings are separated from carcass with cut going from joint of *os coracoides* and *os humerus*. Wing is compound of upper arm, forearm and hand. Breasts are separated with cut coming from dorsal edge of *cartilago xiphoidea*, following the ribs line up to *scapula-humus* joint

where final separated. Thigh and drumstick are separated with cut starting from cranial thigh edge going in pelvis direction, following cranial line. It is separated from the carcass in pelvis joint, going caudal to *os pubis*, and fully divided with circular cut that connects to the start cut. Backs with pelvis remains after wings, breast and drumsticks with thigh are separated (Kralik *et al.*, 2008).

Table 1. Composition and nutritional values of standard chicken feed before supplementation (in %).

Ingredient	Starter	Grower	Finisher
Corn, corngluten	47.05	47.06	54.00
Wheatcornmeal	2.00	3.00	11.00
Wheat	8.30	9.00	3.50
Soybeanmeal, soybeanoil	37.89	36.63	27.06
Lysine	0.29	0.14	0.21
Treonine	0.08	-	0.06
Cholinechloride 60%	0.09	0.06	0.05
Salt, limestone	2.30	2.07	2.05
DL Mhethionin	-	0.23	0.29
MCP-F	1.75	1.26	1.23
Fysal	-	0.30	0.30
PX BRO-S PIPO 0,25%	0.25	-	-
PX BRO-F1 PIPO 0,25%	-	0.25	-
PX BRO-F2 PIPO 0,25%	-	-	0.25
Total	100	100	100
Nutritional values			
Crude protein	22.00	20.50	17.50
Total fat	4.47	5.62	4.00
Linolenic acid	2.31	2.88	2.14
Crudefiber	3.15	3.09	2.87
Amino acids (withoutlysine)	4.22	3.83	3.47
Lysine	1.42	1.24	1.09
Ca	1.05	0.90	0.88
Total P	0.74	0.63	0.61
Exploitable P	-	0.23	0.95
Na	0.14	0.14	0.14
K	0.90	0.86	0.74
Cl	0.22	0.19	0.21
Ash	6.49	5.76	5.43
ME MJ/kg	12.34	12.77	12.64

SOD and GPx blood analysis. Blood samples for superoxide dismutase (SOD) and glutathione peroxidase (GPx) were collected on day 25 and day 42 to establish antioxidant status. SOD and GPx activity was measured spectrophotometrically with automatic analyser SABA 18 (AMS, Italy) using 240 nm wavelength for GSH-Px (RANSEL ®, Randox, UK) and 505 nm wavelength for SOD (RANSOD ®, Randox, UK).

MDA tissue analysis. Lipid peroxidation was established measuring malondialdehyde (MDA) concentration in breast and drumstick meat according to the methods followed by Lemon (1975).

Meat sensory analyses were conducted on breast and drumstick meat according to recommendation for a standardized method of sensory analysis for broilers published by World's Poultry Science Association (WPSA, 1987). Color, structure, juiciness, tenderness, and odor and taste acceptability were assessed using a numerical scale rating quality from 0 (poor) to 8 (excellent), estimated by six panelists for each sample and sensory parameter.

Statistical analysis. All results were processed in Statistic for Windows v.7.1. (2014) software. Basic data processing was performed with standard procedures of descriptive statistic. The results between groups were statistically evaluated using General Linear Models (GLM). Sensory analysis results were processed with *post hoc* Tukey –Kramer HSD test and for other results *post hoc* Fisher test (LSD) is done. Differences were considered as significance at the level of 0.05 or less.

RESULTS AND DISCUSSION

Antioxidative status. The influence of essential oils and their components on biochemical factors in blood is still unclear. SOD and GPx enzymes, both affected by EOs, have important role in cell protection in case of harmful reactive oxygen species (ROS) influence. It is considered that SOD increased activities can serve as a protective mechanism against oxidative stress and lipid peroxidation (Ismail *et al.*, 2013). Blood SOD activity on day 25 was lowest in XT group and highest in XT+ZEO group (Figure 1). Zeolite raised SOD activity up to 25th day. Faix *et al.* (2009) and Polat *et al.* (2011) demonstrated that polyphenol rich EO can raise serum activity of SOD if added up to some dosage limit. If dosage is too high, SOD activity begins to drop relative to EO addition. Within our trial, it is possible that EO component in XTRACT[®] up to day 25 day was below the doses of reaction according to broiler weight, and therefore negatively influenced on SOD activity. Plasma SOD activity became statistically ($P<0.01$) higher in XT than control by day 42. In group where both XTRACT[®] and zeolite added, their combination acted synergistically resulting in constantly higher SOD activity (almost the same on days 25 and 42). Yasar *et al.* (2011) also recorded influence ($P<0.05$) on higher SOD activity when feed herb (*Thymbra spicata*, *Origanum minutiflorum*, *Rosmarinus officinalis*, *Mrytus communis*, *Salvia tomentosa* and ground seed of cumin) levels were from 0.5 to 1.5%. At the end of the trial decreased SOD activity was determined by ZEO addition (ZEO: EX $P<0.05$, ZEO: EX+ZEO $P<0.01$).

XTRACT[®] feed addition showed (Figure 2) significantly lower GPx activity in XT group in relation to ZEO and XT+ZEO group up to day 25. On day 42 results of GPx activity showed differences between groups but with no significance. Contrary, Faix *et al.* (2009) have recorded higher GPx activity connected with cinnamon EO feed addition.

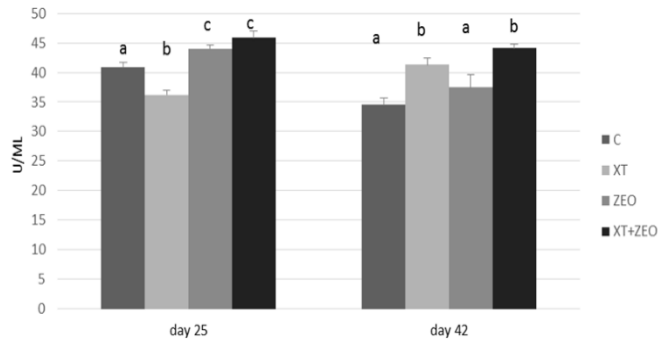


Figure 1. Superoxide dismutase (SOD) activity in broilers' plasma 25th and 42nd day of the trial. C = control; XT = 0.01% XTRACT[®] added; ZEO = 0.2% zeolite added; XT+ZEO = 0.01% XTRACT[®] and 0.2% zeolite added. Means with different superscript letters (a, b) differ significantly ($P<0.05$)

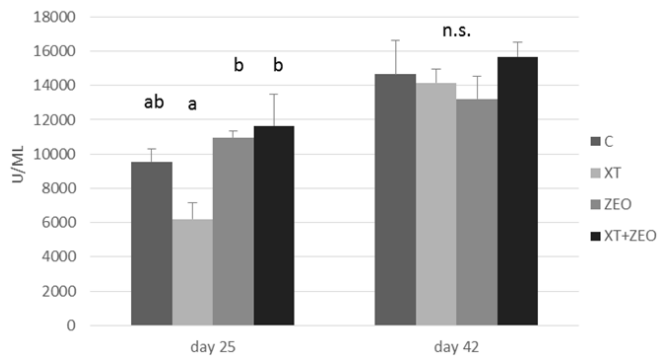


Figure 2. Glutathione peroxidase (GPx) activity in broilers' plasma 25th and 42nd day of the trial. C = control; XT = 0.01% XTRACT[®] added; ZEO = 0.2% zeolite added; XT+ZEO = 0.01% XTRACT[®] and 0.2% zeolite added. Means with different superscript letters (a, b) differ significantly ($P<0.05$)

Malondialdehyde (MDA) is the end product of lipid peroxidation, therefore, monitoring MDA is a useful indicator of the extent of lipid peroxidation in the cell (Khan *et al.*, 2012). In the breast meat (Figure 3) there was significantly lower concentration of MDA in EX and ZEO group related to the control one, lower but without significance in the groups with combined additives. The highest MDA values were found in the control group. The lowest MDA concentration in drumstick meat was found in all experimental groups. Significantly ($P<0.05$) lower MDA concentration were found in ZEO and EX+ZEO group related to the control group (Figure 3). These results are comparable with result Sang-Oh *et al.* (2013) who added cinnamon powder in feed and significantly lowered MDA levels in groups receiving 3%, 5% and 7% supplementation compared to 0% control. On the other hand, Yasar *et al.* (2011) didn't record difference in MDA meat concentration after feeding chicken with herb *Thymbra spicata*, *Origanum minutiflorum*, *Rosmarinus*

officinalis, *Mrytus communis*, *Salvia tomentosa* and ground seed of cumin addition.

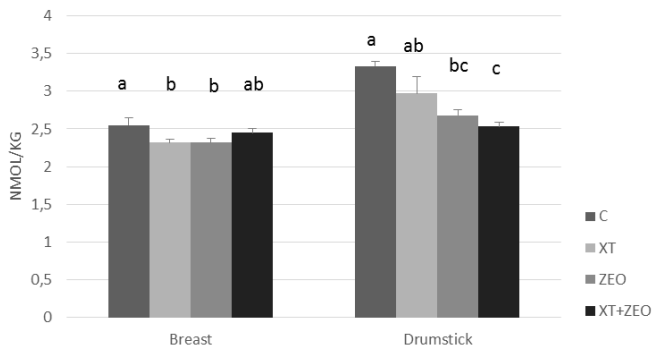


Figure 3. Malondialdehyde (MDA) concentration in breast and drumstick meat of broiler chickens supplemented with XTRACT® and zeolite. C = control; XT = 0.01% XTRACT® added; ZEO = 0.2% zeolite added; XT+ZEO = 0.01% XTRACT® and 0.2% zeolite added. Means with different superscript letters (a, b) differ significantly ($P < 0.05$)

MDA concentration found in meat can be used as an indirect lipid peroxidation indicator. Free radicals coming from unsaturated free fatty acid, which cannot be eliminated by cell, easily oxidize which lead to MDA concentration increase. This principle can be used to get fairly precise data about total enzymatic and non-enzymatic tissue or cell potential (Faix *et al.*, 2009). In our experiment, for both drumstick and breast meat, MDA concentration was lower in all groups where EOs, zeolite or their combination was added, which can indirectly lead to conclusion that they lowered oxidative stress as well.

Broiler growth performance. At the control 25th day of the trial broilers weights were lowest for the control group and highest for the group with XTRACT® added to the diet ($P = 0.07$), which remained by the end of the trial without significances (Table 2). Florou-Paneri *et al.* (2005) who used oregano and Al-Kassie (2009) who added oil extracts of thyme (thymol and carvacrol) and cinnamon

(cinnamaldehyde) are in relation with our results which confirmed higher broilers weights between groups, but not significantly. Al-Kassie *et al.* (2011) described significant influence with red hot chili pepper added in feed (in 0.25%, 0.50%, 0.75% and 1% concentration) on final weight and feed conversion ratio. Several authors (Bravo *et al.*, 2011; Bravo *et al.*, 2014) have confirmed the growth stimulating and feed efficiency effect of the mixture of carvacrol, cinnamaldehyde, and capsicum oleoresin. Bravo *et al.* (2014) explained that the improvement in growth performance with dietary EO is likely a consequence of the increase in dietary net energy for production (NEp). Mansoub (2011) recorded increases in final weight with oregano added feed. Also Sang-Oh *et al.* (2013) recorded higher body weight and significantly higher feed efficacy in group with 5% cinnamon powder added to feed, compared to control, 3% and 7% addition in feed. In contrast to our study, Najafi and Torki (2010) didn't achieve higher final weight although cinnamon EO was added in feed. Zeolite added alone as well as in combination with XTRACT® resulted in slightly higher final weight, which is in similar with results of Mallek *et al.* (2012) who added 1% zeolite in feed and showed no influence on final chicken weight. Zeolite alone had an influence on higher feed consumption ratio in our experiment similar to the Mohebodini (2008) trial. This effect may be due to slowing passage of feed in the intestine, and thereof better feed efficiency. Better feed conversion ratio was reported in many papers when a particular or combinations of EOs were added to feed, especially in the case of oregano and cinnamon (Al-Kassie, 2009; Yasar *et al.*, 2011; Mansoub, 2011; Borazjanizadeh *et al.*, 2011). No negative influences on feed utilization have been noted and only a single trial by Najafi and Torki (2010) reported no influence. In our study feed conversion ratio varied slightly between groups.

After dissection we found significantly higher weight of back in XT and ZEO group ($P < 0.01$), but without differences in group of broilers fed with combination of additives (Table 2). Also heavier drumstick was found in XT and ZEO group in relation to XT+ZEO group.

Table 2. Body weight, feed conversion ratio and cut weight of broilers fed with feed added with XTRACT® and zeolite dietary supplementation, g.

Item	C	XT	ZEO	XT+ZEO	SEM	P-value
BW 25 day	1407.92	1491.66	1438.75	1443.13	11.30	0.069
BW 42 day	2110.42	2181.46	2148.33	2152.71	19.39	0.641
FCR	1.863	1.900	1.857	1.880	0.015	0.813
Weight of different part of broiler carcasses						
Drumstick	266.00 ^{ab}	275.00 ^a	276.75 ^a	262.50 ^b	1.969	0,025
Back	146.32 ^a	157.75 ^b	156.50 ^b	141.58 ^a	1.505	0,000
Breast	626.50	599.50	622.00	603.00	6.961	0,427
Wing	94.41 ^a	95.75 ^a	94.72 ^a	88.86 ^b	0.905	0,031

C = control; XT = 0.01% XTRACT® added; ZEO = 0.2% zeolite added; XT+ZEO = 0.01% XTRACT® and 0.2% zeolite added, BW= body weight; FCR= feed conversion ratio; SEM= standard error of the mean

Meat sensory values. To assess how addition of EO influenced meat color, structure, juiciness, tenderness, odor and flavor acceptability, full sensory analyses was conducted. There are very few literature examples describing the EO influence on sensory property of meat. Lee (2002) considers that because of their quick excretion from organisms, any influence they have would be transitory and therefore is negligible. In our study, breast meat color was negatively influenced with combination of XTRACT® and zeolite, and zeolite alone (Figure 4). A positive influence was observed where XTRACT® was added alone. This may be due to specific green color zeolite as powder has, but the mechanism by which discoloration would occur is unknown. Breast meat structure was rate higher in XT and ZEO groups if compared to control group and XT+ZEO group (Figure 4 and 5). A similar situation was observed with the ratings of drumstick meat structure. The highest influence on juiciness on breast and drumstick meat was seen by XTRACT® and zeolite addition; but breast meat juiciness rated better for XTRACT® and for drumstick juiciness for zeolite addition. Drumstick meat tenderness was most positively influenced XTRACT®, zeolite and their combination; but no influence on breast meat was seen for the combination. Odor and taste acceptability for breast meat and taste acceptability was for drumstick meat improved in all three treatment groups over the control. Mallek *et al.* (2012) also recorded improvement ($P<0.05$) in meat sensory characteristics for drumstick, when 1% zeolite was added in feed. They found more intramuscular n-3 fatty acid in meat. Influences on reduced juiciness, meat structure or flavor intensity were not recorded. Sang-Oh *et al.* (2013) find that overall accessibility index of the taste and flavor of chicken meats was significantly improved in group with 5% cinnamon powder added but not significantly in group with 3% or 7% cinnamon powder added. They concluded that this increase was likely due to the presence of cinnamon powder in muscle tissues of meat.

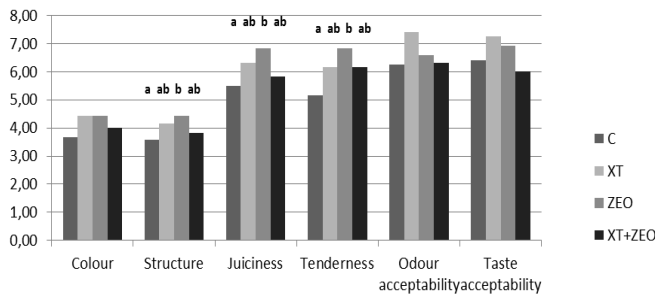


Figure 4. Sensory characteristics of drumstick meat in broiler chickens supplemented with XTRACT® and zeolite. C = control; XT = 0.01% XTRACT® added; ZEO = 0.2% zeolite added; XT+ZEO = 0.01% XTRACT® and 0.2% zeolite added. Means with different superscript letters (a, b) differ significantly ($P<0.05$)

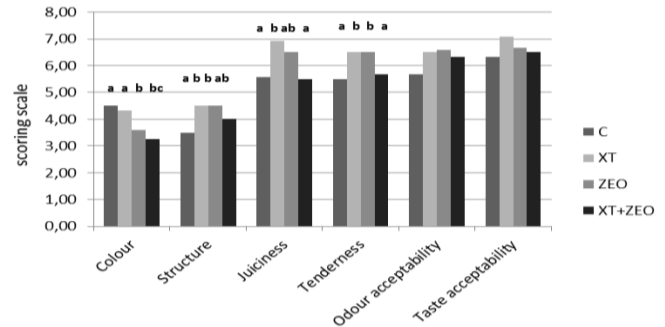


Figure 5. Sensory characteristics of breast meat in broiler chickens supplemented with XTRACT® and zeolite. C = control; XT = 0.01% XTRACT® added; ZEO = 0.2% zeolite added; XT+ZEO = 0.01% XTRACT® and 0.2% zeolite added. Means with different superscript letters (a, b) differ significantly ($P<0.05$)

Conclusions: Significantly lower ($P<0.05$) MDA values in drumsticks meat and GPx values 21th day in ZEO and XT+ZEO group, SOD activity significantly ($P<0.01$) higher in XT than control by day 42 and XT+ZEO on 25th and 42nd day, can serve as a proof for positive effect of these feed additives on antioxidative status of chicken. This can be the reason that final weights were higher in all experimental groups compared to control but with no significance ($P>0.05$), which is hard to develop because of high growing performance characteristic for broiler hybrids. However statistically significant ($P<0.05$) higher weights for backs and drumsticks were observed in experimental groups with XTRACT® (XT) and zeolite (ZEO) addition. Meat sensory characteristics for drumstick and breast meat were improved. Drumstick meat had significantly higher rates for juiciness, tenderness, odor and taste acceptability in experimental groups. Breast meat had significantly higher rates for color, structure, juiciness and tenderness in experimental groups. According to showed results, XTRACT® alone or in combination with zeolite can successfully contribute in chicken fattening in content of better antioxidative status, as well as meat sensory characteristic.

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