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THE EFFECT OF FEEDING MILK THISTLE SEED CAKES ON QUALITY INDICATORS OF BROILER CHICKENS MEAT

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ABSTRACT
This study was conducted to evaluate the effect of feeding milk thistle *(Silybum marianum L.)* seed cakes at dose 5% and 15% in feed mixture on quality indicators of broiler chickens meat. The used milk thistle seed cakes contained 3.73% of flavonolignans and 129.83 mg.kg^{-1} of cyanidin-3-glucoside. A 150 cockerels of Ross 308 were divided into three equal groups. The chickens were fattened on conventional deep litter system. The experimental groups received feed mixtures containing 5% of milk thistle seed cakes (MT5), 15% of milk thistle seed cakes (MT15) and third group was control – without milk thistle seed cakes (C). The trial lasted 37 days. At the end of trial was observed significant higher average weight of chickens (2,320.31 g) in control group. Compare to that the experimental group MT5 achieved significant lower mean bodyweight 2,166.69 g. From the perspective of fattening was decreased growth of chickens where a higher percentage of milk thistle seed cakes (MT15). The group MT15 was up to 420 g lower slaughter weight compared to the control group. This was probably due to the higher content of fiber in the feed. At the end of experiment 15 birds were selected randomly from each group, weighed and slaughtered. Feathers were removed and chickens were eviscerated. Carcass yield was calculated for each group like as percentage of live weight. The MT5 and MT15 group had significantly higher breast meat tenderness that the control group. Initial pH1 was highest in group with its middle addition of milk thistle seed cakes (MT5). Significant differences were not observed between control and group MT15. Breast meat was rated as the best in parameter flavour in control and MT15 group. The thigh meat was evaluated significantly best for colour parameter in MT15 group. Fibreness was rated as the finest in MT15 group. The addition of milk thistle seed cakes do not worsened sensory characteristic of breast or thigh meat of broilers and reflects optimal sensory quality traits.

Keywords: *Silybum marianum L.*; meat quality; growth; texture; colour; flavour

INTRODUCTION
Milk thistle *(Silybum marianum* L.; Asteraceae) have been used for almost 2 000 years as a natural treatment for the liver diseases **(Ding et al., 2001)**. The seeds of milk thistle contain flavonoids (anthocyanins) and flavonolignans (silymarin) in an amount of 1.5 – 3% **(Opletal and Šimerda, 2015)**.

Flavonoids are widespread in nature, including the anthocyanins commonly found in dark-coloured fruits and vegetables. Anthocyanins are produced by plants as secondary metabolites to protect against environmental stress factors and fungal infections **(Chalker-Scott, 1999)**. And they also promote health status **(Wallace, 2011; Pojer et al., 2013)**. Phenolic compounds, mainly anthocyanins have antioxidant and anti-inflammatory activities **(Jung et al., 2014)**. This anthocyanin, cyanidin-3-glucoside (CG) respectively, have been reported to be bioavailable **(Miyazawa et al., 1999)**. CG decreased obesity and circulating triglycerides in an in vivo study **(Wei et al., 2011)**. In vitro, CG decreased inflammation in isolated vascular endothelial cells and monocytes and produced an insulin-like effect in human adipocytes **(Luo et al., 2012; Scazzocchio et al., 2011)**.

The main active substances occurring in milk thistle are flavonolignans, which are hepatoprotective substances. The mixture of silydianin (10%), silychristin (20%), silybin A and silybin B (50 – 60%), isosilybin A and isosilybin B is known as silymarin **(Opletal and Skřivanová, 2010; Ding et al., 2001; Zahid and Durrani 2007; Comelli et al., 2007)**. Silymarin complex exhibits chemopreventive activity against chemical, viral, bacterial and fungal toxins, inhibits lipid peroxidation, and stabilizes the cell membranes of the liver parenchyma **(Opletal and Skřivanová, 2010)**. Various trials showed that silymarin addition in diet or silymarin administration increased productive and reproductive performances and improved livestock health status of animals **(Tedesco, 2001)**.

Many works investigate the effect of herbs addition to feed mixtures for broiler chickens and their influence to the meat quality **(Haščík et al., 2015; Bobko et al., 2009)**.

The rapid growth of modern broilers hybrid and toxic substances in the feed mixtures can lead to metabolic and oxidative stress. It can worsen feed conversion ratio, growth parameters and it can affect the quality of chicken meat **(Erdogan et al., 2005; Carreras et al., 2004)**. The consumers have very high requirements on their food. It must be natural, healthy, quality, safe and, on top of that, it should have pleasant appearance, texture, odour and taste **(Drobná et al., 2006)**.

This study was conducted to evaluate the effect of feeding milk thistle seed cakes at dose 5% and 15% in feed mixture on quality indicators of broiler chickens meat.

MATERIAL AND METHODOLOGY

Growth performance, body and chemical composition

The used milk thistle seed cakes contained 3.73% of flavonolignans and 129.83 mg.kg^{-1} of cyanidin-3-glucoside. Table 1 shows chemical composition of used milk thistle seed cakes.

Table 1 Chemical composition of milk thistle seed cakes.

Dry matter (%)	100
Gross energy (MJ.kg^{-1})	17.44
Crude protein (%)	18.65
Crude fat (%)	8.66
Crude fibre (%)	25.13
Crude ash (%)	5.84

The experiment was performed with cockerels of Ross 308 hybrid (n = 150) which were fattened on conventional deep litter system. Wood shavings were used as bedding material. The trial was conducted from day 12 to day 37 of chick's age. Room temperature and humidity were controlled. Lighting system was 16 hours light and 8 hours dark. Cockerels were divided into three equal groups. The two experimental groups received feed mixtures containing 5% and 15% of milk thistle seed cakes (groups MT5 and MT15, respectively). The third group was without milk thistle seed cakes (Control group). The rations were calculated according to the Recommended nutrient content in poultry diets and nutritive value of feeds for poultry **(Zelenka et al., 2007)**. The composition of feed mixtures is shown in Table 2.

The chickens were fed *ad-libitum*. Health status was evaluated daily and live weight measured every week during the trial. Body weight gain was measured individually.

At the end of experiment fifteen birds were selected randomly from each group, weighed and slaughtered. Feathers were removed and chickens were eviscerated. Carcass yield was calculated. The breast muscle and leg muscle were deboned and weighed in these selected chickens. These values were calculated by the percentage of live weight.

Table 2 Composition of feed mixture (g.kg^{-1}).

Component	C	MT5	MT15
Wheat	378.2	271.8	269
Corn	247	282.4	251
Milk thistle seed cakes	0	50	150
Soybean meal	105	120	128
Soybean extruded	190	190	78
Rapeseed oil	20	30	40
Wheat gluten	18.8	15.2	40
Premix*	30	30	30
Monocalciumphosphate	7	6.5	7
Limestone milled	4	4	5
L-lysine	0	0	2
Chemical composition (per kg of diet)			
Dry matter (%)	100	100	100
Gross energy (MJ)	18.59	18.83	19.07
Crude protein (%)	21.41	21.73	22.43
Crude fat (%)	7.60	9.60	8.96
Crude fibre (%)	2.81	4.07	7.03
Crude ash (%)	5.96	5.84	6.65

* Premix contains (per kg): lysine 60 g; methionine 75 g; threonine 34 g; calcium 200 g; phosphorus 65 g; sodium 42 g; copper 500 mg; iron 2,500 mg; zinc 3,400 mg; manganese 4,000 mg; cobalt 7 mg; iodine 30 mg; selenium 6 mg; tocopherol 450,000 mg; calciferol 166,700 IU; tocoferol 1,500 mg; vit K 350 mg; B_1 140 mg; B_2 230 mg; B_6 200 mg; B_{12} 1,000 mg; biotin 7 mg; niaciamid 1,200 mg; folic acid 57 mg, calcium pantothenate 450 mg; choline chloride 6,000 mg; salinomycin sodium 2,333 mg.

Texture, colour and pH of meat

The tenderness of the fillets was determined through the application of the Meullenet–Owens razor shear (MORS) test, using a texture analyzer (Model TA-XT2*Plus*, Texture Technologies, Scarsdale, N.Y., U.S.A.) as described by **Meullenet et al., (2004)** and **Cavitt et al., (2005)** during which Razor Blade Shear Force (N) were recorded. Tests using the MORS blade are conducted on whole intact right fillets with 5 replicates. The sharp blade was replaced every 80 measurements for optimum shearing performance. Test Settings: test speed 10 mm.s^{-1}, distance 20 mm.

Colour measurement was performed by CIE L*a*b* colour space. L* (lightness), a*(redness) and b* (yellowness) values from the breast muscle sample surface on the dorsal side were measured using a Spectrophotometer CM-3500d (Konica Minolta Sensing Inc., Osaka, Japan) in SCE mode (specular component excluded), angle 8°, 8 mm slit. Each sample was measured at three places 1-hour *post-mortem*. Average value was taken as the final result. ΔE^*_{ab} **(CIE, 2007)** was calculated according next formulas **(Valous et al., 2009)**:

$$\Delta E^*_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

$$\Delta L^* = L^*_{control} - L^*_{group}$$

$$\Delta a^* = a^*_{control} - a^*_{group}$$

$$\Delta b^* = b^*_{control} - b^*_{group}$$

The samples was measured using pH meter Portavo 907 Multi (Knick Elektronische Messgeräte GmbH & Co. KG, Berlin, Germany) with a needle-type electrode (SE104N; Knick Elektronische Messgeräte GmbH & Co. KG, Berlin, Germany) immediately (inicial pH, abbreviation pH1) after chicken´s slaughter and 1 hour *post-mortem* (abbreviation pH2).

Sensory analysis

Sensory analysis of breast and thigh muscle samples was evaluated by 10 panellists in special sensory laboratory (Department of Food Technology, MENDELU) according ISO 8589. Each sample (breast and thigh) was packed into plastic case and frozen (freezer, -18 °C). After two weeks was thawed (cold storage room, 4 °C) and boiled in convection oven (200 °C, 60% humidity, 1 hour). Professional evaluation group was represented by a panel of trained panellists under ISO 8586-1. We used a graphic non-structured scale (100 mm) to compare experimental group of descriptors (odour, colour, fibreness, chewiness, juiciness, flavour, fatty taste) with control group.

Statistical analysis

Data has been processed by Microsoft Excel (USA) and Statistica version 12.0 (CZ). We used one-way analysis (ANOVA). To ensure evidential differences Scheffe´s test was applied and p <0.05 was regarded as statistically significant difference.

RESULTS AND DISCUSSION

Growth performance, body and chemical composition

At the end of trial was observed significant (p <0.05) higher average weight of chickens (2,320.31 g) in control group. Compare to that the experimental group MT5 achieved significant lower mean bodyweight 2,166.69 g. The significant lowest mean bodyweight was achieved in the group MT15 with value 1,988.78 g at the end of trial (Table 3).

Table 3 Live weight at the day of slaughter (g).

Group	Mean ±standard error
C	2,320.31 ±29.24^{c}
MT5	2,166.69 ±36.43^{b}
MT15	1,988.78 ±30.09^{a}

^{a,b,c} – different letters are statistically significant differences (p <0.05).

According to the technological procedure for ROSS 308, the average body weight of cockerels would be 2,493 g at 37 days of age **(Aviagen Group, 2014)**. This is much closer to the value of the control group (2,320 g) in our trial. **Suchý et al., (2008)** in their experiment observed then the addition of 0.2% and 1% *Sylibum Marianum* seed cakes caused a decrease in the weight gain. **Gawel et al., (2003)** found an increase in the slaughter weight in broilers when supplied silymarin.

From the perspective of fattening was decreased growth of chickens where a higher percentage of milk thistle seed cakes (MT15). The group MT15 was up to 420 g lower slaughter weight compared to the control group, which was probably due to the higher content of fiber in the feed.

Table 4 present the carcass yield parameters of chickens. The carcass yield was not show significant (p >0.05) differences. Carcass yield stated in the technological procedure for ROSS 308 **(Aviagen Group, 2014)** is 72.08% for 2,200 g live weight. The highest carcass yield showed the control group with a value 72.09%. It is comparable with technological procedure for ROSS 308 **(Aviagen Group, 2014)**.

The higher breast meat yield was found in the group 5% of milk thistle seed cakes (22.11 ±0.42% SE). The differences among groups were not statistically significant (p >0.05). The manual for hybrid Ross 308 **(Aviagen Group, 2014)** is stated similar percentage of breast muscle

Table 4 Body composition (%).

Group	n	Carcass	Breast meat	Leg meat
		Mean ±standard error		
C	15	72.09 ±1.05	21.62 ±0.63	14.84 ±0.33
MT5	15	71.44 ±0.95	22.11 ±0.42	14.77 ±0.28
MT15	15	70.51 ±0.75	20.70 ±0.49	15.21 ±0.37

Differences between groups are not signifficant (p >0.05).

Table 5 Chemical analysis of breast and thigh (%).

		n	C	MT5	MT15
			Mean ±standard error		
Dry Matter	Breast meat	6	23.97 ±0.62	24.20 ±0.25	23.56 ±0.54
	Leg meat		24.62 ±0.37	24.12 ±0.29	23.95 ±0.18
Crude Protein	Breast meat	6	20.94 ±0.77	21.39 ±0.28	21.68 ±0.57
	Leg meat		18.68 ±0.19	18.46 ±0.22	18.89 ±0.31
Crude Fat	Breast meat	6	1.24 ±0.19	1.10 ±0.11	0.96 ±0.15
	Leg meat		4.17 ±0.25	4.31 ±0.28	3.88 ±0.34

Differences between groups are not signifficant (p >0.05).

Table 6 Effect of addition milk thistle seed cakes into feed on texture, pH and colour of breast meat (means ±SE).

Parameter	Control	MT5	MT15
Razor Blade Shear [N]	11.23 ±0.47[a]	8.94 ±0.27[b]	9.52 ±0.36[b]
L*	62.00 ±2.20[a]	61.25 ±1.82[a]	62.58 ±2.07[a]
a*	4.42 ±0.35[a]	4.86 ±0.71[a]	5.16 ±0.87[a]
b*	11.33 ±0.44[a]	13.19 ±0.62[b]	13.51 ±0.27[b]
ΔE^*_{ab}	0[a]	2.06[a]	2.38[a]
pH1	6.40 ±0.08[a]	6.64 ±0.09[b]	6.47 ±0.12[a]
pH2	6.12 ±0.10[a]	6.38 ±0.11[b]	6.33 ±0.20[ab]

pH1 values were measured just after slaughter in breast, likewise pH2 values were measured after 1 hour post-mortem.
ΔE^*_{ab} is compared with control group.
[a,b] Means in a row within effect with no common superscript differ significantly (p <0.05).

of body weight to our results.

The highest non-significant difference (p >0.05) in thigh meat yield was observed in the MT15 group (15.21 ±0.37%) compared to the experimental groups. The manual for the hybrid Ross 308 **(Aviagen Group, 2014)** indicates a yield of leg meat 16.03% for 2,200 g of live weight.

Schiavone et al., (2007) performed an experiment with the addition of silymarin into feed mixture for broilers chickens. They found that the control group (without silymarin) achieved significantly highest carcass yield 75.04%. The breast muscle was reached highest weight (29.62%) in group with addition of 40 ppm of silymarin. And the leg muscle reached the highest yield (29.34%) in the group with addition of 80 ppm of silymarin. The lipid content of breast (1.19%) and thigh (3.81%) muscle was affected (p <0.05) by silymarin supplementation, and the lowest amount of lipid content was observed in group with 40 ppm of silymarin.

The chemical composition of breast and thigh muscles is shown in the Table 5. Differences between groups are not significant (p >0.05).

Chemical composition of breast and thight muscles of chickens was not found statistically significant differences. The breast meat of MT15 group contain the most of protein and minimum of fat. The nutrient composition of leg muscle is comparable across all three groups.

Table 7 Sensory analysis of breast meat (mm).

Group		C	MT5	MT15
		Mean ±standard error		
Sensory trait	n	60	60	60
Odour		63.97 ±2.75[a]	70.80 ±1.75[a]	69.27 ±1.60[a]
Colour		73.18 ±1.45[a]	75.50 ±1.71[a]	77.40 ±1.39[a]
Fibreness		55.18 ±2.61[a]	56.43 ±2.38[a]	56.97 ±2.06[a]
Chewiness		62.75 ±2.51[a]	59.78 ±3.05[a]	58.60 ±2.20[a]
Juiciness		51.22 ±2.77[a]	44.03 ±3.08[a]	47.33 ±2.07[a]
Flavour		74.00 ±1.50[b]	65.02 ±3.02[a]	73.97 ±1.73[b]
Fatty taste		78.77 ±2.09[a]	81.08 ±1.39[a]	83.40 ±1.06[a]

[a,b] – different letters on one line - statistically significant differences (p <0.05).

Table 8 Sensory analysis of thigh meat (mm).

Group		C	MT5	MT15
		Mean ±standard error		
Sensory trait	n	60	60	60
Odour		70.98 ±1.94[a]	69.07 ±1.92[a]	72.02 ±1.42[a]
Colour		50.08 ±1.19[a]	53.05 ±1.51[ab]	57.72 ±1.52[b]
Fibreness		56.67 ±1.39[a]	61.58 ±1.46[b]	65.55 ±1.24[b]
Chewiness		64.83 ±1.59[a]	65.47 ±1.60[a]	66.13 ±1.35[a]
Juiciness		66.90 ±1.97[a]	64.30 ±1.58[a]	67.70 ±1.54[a]
Flavour		74.25 ±1.87[a]	69.42 ±2.16[a]	72.78 ±1.72[a]
Fatty taste		76.35 ±2.56[a]	77.15 ±2.62[a]	79.13 ±2.21[a]

[a,b] – different letters on one line - statistically significant differences (p <0.05).

Texture, colour and pH of meat

The Razor Blade Shear Force results (n = 30) are shown in Table 7. The MT5 and MT15 groups had significantly (p <0.05) higher breast meat tenderness that the control group. Presented in Table 6.

The colour change is not significant in all coordinates (lightness L*, a* and b*), see Table 6. There were no significant differences between all three groups. However, compared with the control group, the yellowness (b*) was higher in both experimental groups. The total colour change (ΔE^*_{ab} from 1.5 to 3.0) is clearly perceptible but not yet discordant and it is acceptable for consumers **(Saláková, 2012)**.

The pH values from control group and groups with addition of *Silybum marianum* into feed is illustrated in Table 6. Initial pH1 was highest (p <0.05) in group with its middle addition of milk thistle seed cakes (MT5). Significant differences were not observed between control and group MT15. The highest pH decrease was noticed in control group breasts. pH2 values measured after 1-hour post mortem is obviously higher in MT5 and MT15 than control group. Between control and MT5 group was significant difference (p <0.05). Some authors **(Zhang et al., 2011, Salami et al., 2015)** confirm the slower post mortal process of muscle acidification on the grounds of various feeding supplementation, but still with acceptable sensory traits.

Sensory analysis

Breast meat was rated as the best in parameter flavour in control and MT15 group (p <0.05). The odour parameter was the best evaluated in MT5 group. Chewiness and juiciness were the best rated in the control group. The fibreness parameter was best rated in the group with the highest addition of milk thistle seed cakes (MT15). This data was not significant (p >0.05) (Table 7).

Some people prefer to consume leg meat of chickens, because it´s more fatty and therefore contains more of flavour substances **(Komprda et al., 2002)**. Table 8 shown sensory analyses of thigh meat. The leg meat was evaluated significantly (p <0.05) best for colour parameter in MT15 group. Fibreness was rated as the finest in MT15 group (p <0.05). The most typical flavour of chicken meat was evaluated in the control group and the chewiness parameter was the best in MT15. There are no significant differences.

Overall assessment of sensory analysis of breast and leg meat shows that the flavour was the best evaluated in the control group. The color and fibrous parameters of meat were the best in the MT15 group. The fatty taste was the lowest in the control group.

Cook and Homer (1996) classified chewiness, juiciness and flavour intensity as the important sensory traits in sensory analyses. This claim is also confirms **Poste et al., (1996)** who advise that flavour is one of the most important sensory traits.

CONCLUSION

The MT5 and MT15 group had significantly (p <0.05) higher breast meat tenderness that the control group. Overall, the total colour change of meat is not significant differences between all three groups. Initial pH1 was highest (p <0.05) in group with its middle addition of milk thistle seed cakes (MT5). Significant differences were not observed between control and group MT15. Breast meat was rated as the best in parameter flavour in control and MT15 group (p <0.05). The leg meat was evaluated significantly (p <0.05) best for colour parameter in MT15 group. Fibreness was rated as the finest in MT15 group (p <0.05).

In this study, the presence of milk thistle seed cakes at dose 5% and 15% in feed mixture were evaluated. The addition of this do not worsened sensory characteristic of breast or leg meat of broilers and reflects optimal sensory quality traits.

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