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Glycerol as source of energy in broiler chicken fattening

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Abstract

The objective of this study was to verify the possibility of replacing soybean oil in a diet with glycerol, and investigate the effect of glycerol on performance indicators and health in broiler chickens. The experiment was performed on 122 one-day-old chickens that were divided based on sex into two control groups (30 females and 31 males) and two experimental groups (30 females and 31 males). Half (50%) of the soybean oil in diets used in the experimental groups was replaced with glycerol at a ratio of 1:2. On 15, 32 and 38 day of age chickens of both sexes in the experimental group that were fed with diets containing glycerol showed significantly higher ($p \leq 0.01$) mean body weight compared to the control group. At the end of the experiment, the mean weight of chickens in the control group was 2.078 kg, whereas the mean weight of chickens in the experimental group was 2.341 kg. In females, the overall consumption of diets within 38 days of fattening was 3.588 kg in the control group and 4.011 kg in the experimental group, in males, it was 3.915 kg in the control group and 4.366 kg in the experimental group, i.e. it was higher in experimental chickens. Feed conversion in chickens in experimental groups was better, being 1.84 kg in the control group and 1.81 kg in the experimental group in females, and 1.73 kg in the control group and 1.72 kg in the experimental group in males. It follows from our results that the optimum amount of glycerol in feed for poultry is 5%.This study presents an original solution to optimize feed formula by replacing plant oil with glycerol. The results of the study can improve production indicators and economy in broiler fattening.

Rapeseed methylester, amino acids, fatty acids, gross energy, performance indicators

The gradually increasing biodiesel production (rapeseed oil methylester) poses a problem of the utilization of glycerol as a by-product of this technology. Since the amount of glycerol generated during the production of methylester exceeds the demands of industry (e.g., chemical industry), new alternatives for its use are currently being sought. Animal production seems to be a promising field to use glycerol as a source of energy in relatively large amounts, ensuring the optimum way of its recycling at the same time.

The use of glycerol in the nutrition of farm animals was first mentioned in the 1960s and 1970s. For example, some of the studies investigated the effect of dietary glycerol on reproduction of sows and on the composition of fatty acids in lipids in fat tissue (Neville et al. 1970; Demarne et al. 1977), or reported the use of glycerol in poultry nutrition and its effect on reproduction (Neville et al. 1971; Westfall and Howarth 1976). The use of glycerol in feeds for chickens was reported by Renner and Elcombe (1964), and by Carew and Foss (1974). Emmanuel et al. (1983) studied the effect of glycerol on the synthesis of glucose in fattened chickens whereas Lin et al. (1976) investigated the effect of glycerol on the activity of lipogenic enzymes and the synthesis of fatty acids.

According to most authors, the optimum content of glycerol in diets for broiler chickens is in the range of 5-10% (Simon et al. 1996; Barteczko and Kaminski 1999; Cerrate et al. 2006). When included at this amount, crude glycerol showed a positive effect on performance characteristics, with no negative effect being observed on feed conversion. The dose of 25% of glycerol was associated with pathological changes in organs and had a negative effect on performance indicators in broiler chickens (Simon et al. 1997). Dozier

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et al. (2008) supplemented the diet for broiler chickens with 3, 6 and 9% of glycerol in individual phases of the fattening period, and monitored not only performance indicators (feed intake, body weight) but also the intake and excretion of energy and nitrogen. Their results showed that the apparent metabolizable energy (ME) of glycerol was effectively utilized by broiler chickens and that glycerol could be used as a source of energy in the diets for broiler chickens. However, what still has to be specified is the acceptable content of residual methanol that originates from the separation of fatty acids in biodiesel production (Cerrate et al. 2006). One positive finding is that glycerol improves technological and hygienic quality of granules, as reported by Schröder and Südekum (1999). Glycerol doses above 100 g per kg of feed may cause technological problems in the granulation of diets.

The aim of this study was to verify the possibility of replacing 50% of soybean oil with glycerol in a diet at a ratio of 1:2, and to examine the effect of glycerol on performance indicators and health in broiler chickens.

Materials and Methods

The comparative feeding trial was performed on meat type ROSS 308 hybrid of broiler chickens in the accredited experimental enclosure of the Department of Nutrition, Animal Husbandry and Animal Hygiene, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Brno. The experiment was conducted on 122 one-day-old chickens that were divided (according to sex) to two control groups C1 and C2 (30 females and 31 males) and two experimental groups G1 and G2 (30 females and 31 males). Chickens were kept and fed in compliance with the technological procedure for ROSS 308 combination hybrid chickens, on deep bedding, at a density of 1 chicken per 0.25 m^2. Chickens were fed three kinds of diets in form of mash during the experiment: pre-fattening diet (BR 1) from 1–14 days of age, fattening diet from 15–31 days of age (BR 2), and post-fattening diet from 31 to 38 days of age (BR 3). The feed was administered in self-feeders; water was provided from hat-shaped waterers, both were available *ad libitum*.

Diets were analysed prior to the feeding trial. Dry matter was determined gravimetrically by drying the sample at required conditions (105 ºC). The content of nitrogen was determined by the Kjeldahl method using the Buchi analyser (Centec automatika, spol. s.r.o.). The content of crude protein in the feed was determined by multiplying the result by a coefficient of 6.25. The content of crude fat was determined by extraction using ANKOM^{XT10} Fat Analyser (O.K. Servis BioPro, Czech Republic). Crude fibre was determined using ANKOM^{220} Fiber Analyser (O.K. Servis BioPro). Ash content was determined gravimetrically after the burning of a sample at a temperature of 550 °C at defined conditions. Calcium, phosphorus, and magnesium were determined by combustion and extraction followed by titration. The content of nitrogen free extractives (NFE) was calculated.

The amino acid spectrum was determined after acid hydrolysis of the feed sample with 6 N HCl, at 110 °C for a period of 24 h, using AAA 400 (INGOS a.s. Praha, Czech Republic) automatic amino acid analyser on the basis of colour reaction between amino acids and an oxidation reagent (ninhydrin). In order to evaluate the quality of fat, gas chromatography was carried out to determine the contents of individual fatty acids using a GC 2010 Shimadzu Gas Chromatograph (Shimadzu, Japan). The extraction of fat from meat for determining the composition of fatty acids, especially PUFAs, was performed according to Hara and Radin (1978). The determination of heat of combustion ($MJ \cdot kg^{-1}$) was determined by calorimetry, combusting the feed sample in a bomb calorimeter in oxygen atmosphere, using AC 500 (Leco, USA).

Diets were prepared from the basic diet according to the procedure described below, in the Institute's feed mixing facility. In diets intended for experimental groups of chickens, 50% soybean oil was replaced with glycerol at a ratio of 1:2; one portion of soybean oil was replaced with two portions of glycerol. Pure glycerol (E 422) contains 98% of glycerol in dry matter. It is an additive as defined in Regulation No. 318/2003 (Anonymous 2003). Pure glycerol used in the experiment provided 15.3 $MJ \cdot kg^{-1}$ GE.

Control and experimental diets (50 kg) were prepared from the basic diet. The control diet for pre-fattening (BR1) was prepared using 48.50 kg basic diet and 1.5 kg soybean oil, the experimental diet for pre-fattening included 47.75 kg basic diet, 0.75 kg soybean oil and 1.5 kg glycerol. The control diet for fattening (BR2) was prepared from 48.20 kg basic diet and 1.8 kg soybean oil, whereas the experimental diet for fattening included 47.30 kg basic diet, 0.9 kg soybean oil and 1.8 kg glycerol. The control diet for post-fattening (BR3) was prepared from 47.80 kg basic diet and 2.2 kg soybean oil, the experimental diet for post-fattening was prepared from 46.70 kg basic diet, 1.1 kg soybean oil and 2.2 kg glycerol.

In the course of the experiment, the growth intensity in broiler chickens was monitored by weighing individual chickens at 1, 15, 32 and 38 days of age. Mean daily weight gain was calculated for the individual phases of fattening and for the whole period of fattening. The consumption of diet was monitored daily and feed conversion was calculated. Throughout the whole period of fattening, the health condition of chickens was examined and the mortality rate was recorded.

Results were processed using mathematical and statistical methods implemented in UNISTAT for Excel, version 56, and using the Tukey-HSD multiple comparison test. Differences between the mean values were tested at the level of significance being $p \leq 0.01$ and $p \leq 0.05$.

Results

The substitution of soybean oil with glycerol resulted in changes in the contents of nutrients in diets (Table 1). In experimental diets (G), the content of NNES and NaCl increased whereas the content of total protein decreased, particularly in the diet BR 2 and BR 3; the content of crude fat and starch also decreased. The replacement of soybean oil with glycerol (the G group) resulted in a slight decrease in the contents of most amino acids (AA) which corresponded with the finding of a lower content of total protein in experimental diets. The BR 1 diet showed minimum differences in the amino acid spectrum, which is most likely attributed to a common and permissible analytical error. When the content of glycerol in diets was higher, the differences increased.

Table 1. Changes in the contents of nutrients in diets for chickens after the replacement of soybean oil by glycerol ($g \cdot kg^{-1}$ in 100% dry matter)

	BR1		BR2		BR3	
	Control	Experiment	Control	Experiment	Control	Experiment
Ca	11.18	11.39	9.95	9.96	9.95	9.96
P	8.61	8.77	7.72	7.44	7.72	7.44
Mg	2.72	2.72	2.74	2.75	2.74	2.75
NaCl	2.6	5.9	2.9	6.0	2.9	6.0
CP	258.4	257.4	245.9	240.3	245.9	240.3
Crude fat	51.6	36.5	58.0	39.0	58.0	39.0
Crude fibre	24.3	24.1	26.3	27.5	26.3	27.5
NFE	599.9	613.5	609.9	633.2	609.9	633.2
Starch	411.7	394.6	415.0	400.6	415.0	400.6
Ash	65.8	68.4	59.8	59.9	59.8	59.9
ME ($MJ \cdot kg^{-1}$)	12.9	12.4	13.3	12.3	13.3	12.3
GE ($MJ \cdot kg^{-1}$)	18.99	18.6	19.1	18.7	19.1	18.7

BR1 – diet for pre-fattening, BR2 – diet for fattening, BR3 – diet for post-fattening, CP – crude protein, NFE – nitrogen-free extractives, ME – metabolisable energy, GE – gross energy

The replacement of soybean oil with glycerol resulted in qualitative changes in the contents of fatty acids (FAs) between control and experimental diets, as documented in Table 3. In the BR 1 experimental diet, the total content of FAs decreased to 67.78% (i.e. by 32.22%) compared to the BR 1 control diet. This decrease in the total content of FAs was associated with a decrease in individual FAs ranging from 16.98% (lauric acid, C12:0) to 43.55% (caprylic acid, C 8:0). In the BR 2 experimental diet, the total content of FAs decreased to 66.22% (i.e. by 33.78%) compared to the BR 2 control diet. The overall decrease in the total content of FAs was associated with a decrease in individual FAs from 18.50% (lauric acid, C12:0) to 39.06% (erucic acid, C22:1n9). In the BR 3 diet, the total content of FAs dropped by 39.85% compared to the BR 3 control diet. Reduction in the total content of FAs was also associated with a decrease in the contents of individual FAs, ranging from 4.57% (caproic acid, C6:0) to 45.02% (myristic acid, C14:0).

The development of body weight in chickens (according to sex, throughout the experiment) is given in Table 4. On days 15, 32 and 38 of fattening, the mean body weight in chickens that were fed diets containing glycerol was significantly higher compared to the control group. Differences between group C and group G in the body weight of chickens on

Table 2. Changes in the qualitative composition of amino acids in diets for chickens after the replacement of soybean oil with glycerol (100% dry matter)

	BR1		BR2		BR3	
Amino acid	Control	Experiment	Control	Experiment	Control	Experiment
Aspartic acid	23.36	23.41	20.89	20.47	19.00	17.71
Threonin	10.17	9.41	8.13	8.26	8.14	7.15
Serin	11.85	11.09	10.16	10.07	9.73	9.31
Glutamic acid	46.27	45.70	43.69	43.43	42.18	38.71
Proline	12.12	11.90	11.81	11.81	13.68	12.83
Glycine	9.95	9.75	9.03	8.93	8.93	7.95
Alanine	9.50	9.41	8.47	8.48	8.37	7.61
Valine	12.52	12.43	11.40	11.20	10.06	9.42
Methionine	5.81	6.27	4.97	4.86	4.30	3.75
Isoleucine	9.83	9.63	8.92	8.82	8.48	7.95
Leucine	20.34	20.39	18.52	18.66	16.62	15.21
Tyrosine	7.38	8.18	7.00	7.46	6.45	6.02
Phenylalanine	11.73	11.76	10.39	10.63	10.29	8.97
Histidine	7.49	7.51	6.55	6.90	5.54	5.68
Lysine	15.98	16.02	13.44	13.23	11.20	11.47
Arginine	18.44	18.37	16.48	16.29	15.15	14.98
Total	232.74	231.23	209.85	209.50	198.12	184.72

BR1 – diet for pre-fattening, BR2 – diet for fattening, BR3 – diet for post-fattening

Table 3. Changes in the contents of fatty acids in diets for chickens after the replacement of soybean oil with glycerol

	BR1		BR2		BR3	
Fatty acids	Control	Experiment	Control	Experiment	Control	Experiment
Capronic acid (C6:0)	0.002	0.002	0.002	0.001	0.002	0.002
Caprylic acid (C8:0)	0.002	0.001	0.002	0.001	0.001	0.001
Lauric acid (C12:0)	0.008	0.007	0.007	0.006	0.005	0.003
Tridecanoic acid (C13:0)	-	-	-	-	0.0006	0.0004
Myristic acid (C14:0)	0.040	0.026	0.049	0.037	0.049	0.027
Palmitic acid (C16:0)	4.901	3.440	5.299	3.717	5.797	3.736
Palmitoleic acid (C16:1)	0.053	0.040	0.065	0.049	0.068	0.039
Heptadecanoic acid (C17:0)	0.044	0.028	0.046	0.031	0.053	0.033
Stearic acid (C18:0)	1.356	0.900	1.523	0.930	1.723	0.967
Oleic acid (C18:1n9t)	12.744	8.416	14.663	9.167	15.833	9.078
Linoleic acid (C18:2n6c)	19.615	13.479	21.291	14.567	23.265	14.327
γ-linolenic acid (C18:3n6)	2.277	1.464	2.486	1.592	2.607	1.490
Arachidic acid (C20:4n6)	0.182	0.126	0.211	0.129	0.198	0.121
cis-11-eicosenoic acid (C20:3n3)	0.179	0.121	0.207	0.141	0.201	0.117
Behemic acid (C22:0)	0.168	0.132	0.213	0.137	0.241	0.162
Erucic acid (C22:1n9)	0.030	0.020	0.039	0.024	0.034	0.020
Total fatty acids	41.601	28.198	46.104	30.528	50.085	30.129

BR1 – diet for pre-fattening, BR2 – diet for fattening, BR3 – diet for post-fattening

15, 32 and 38 day of fattening were highly significant ($p \leq 0.01$). Differences in the mean weight gain in the period from day 1 to 15 of fattening and from day 1 to 38 of fattening were highly significant ($p \leq 0.01$), in both males and females.

Table 4. Mean body weight of chickens in the course of the experiment

Age (day)	Group	Total (g ± SD)	*P*	Females (g, ± SD)	*P*	Males (g, ± SD)	*P*
1	Control	43 ± 0.003	NS	42 ± 0.003	NS	43 ± 0.003	NS
	Experiment	42 ± 0.003		43 ± 0.004		43 ± 0.003	
15	Control	326 ± 0.09	≤ 0.01	315 ± 0.09	≤ 0.01	336 ± 0.09	≤ 0.01
	Experiment	433 ± 0.07		440 ± 0.04		425 ± 0.08	
32	Control	1521 ± 0.33	≤ 0.01	1438 ± 0.27	≤ 0.01	1604 ± 0.37	≤ 0.01
	Experiment	1735 ± 0.21		1642 ± 0.09		1828 ± 0.25	
38	Control	2078 ± 0.40	≤ 0.01	1929 ± 0.31	≤ 0.01	2228 ± 0.42	≤ 0.01
	Experiment	2341 ± 0.28		2184 ± 0.12		2498 ± 0.30	

(g) (mean ± SD), NS – non-significant difference

Feed consumption is one of the basic economic criteria for evaluation of fattening (Table 5). The total consumption of a diet within 38 days of fattening was 3.588 kg (C) and 4.011 kg (G) in female chickens, and 3.915 kg (C) and 4.366 kg (G) in male chickens. The body weight of experimental chickens that were fed the diet containing glycerol increased by 423 g in females and by 451 g in males compared to control. In the period of fattening (38 days), nett production of chickens (after subtracting the weight of one-day-old chickens) was 58.50 kg (C) and 67.71 kg (G) in females and 68.57 kg (C) and 78.57 kg (G) in males. The consumption of diets in experimental groups was higher, which was also associated with lower feed conversion in experimental groups: 1.84 kg (C) and 1.81 kg (G) in females, and 1.73 kg (C) and 1.72 kg (G) in males.

Chickens in both groups did not show any clinical symptoms of diseases throughout the experiment, and no mortality was recorded.

Table 5. Feed consumption and conversion in groups of chickens in individual stages of fattening

	Consumption of diet				Conversion of diet			
	Period (days)				Period (days)			
	1 - 15	15 - 32	32 - 38	1 - 38	1 - 15	15 - 32	32 - 38	1 - 38
Females								
Control	13.15	65.00	29.50	107.65	1.55	1.46	1.94	1.84
Experiment	17.20	73.30	33.85	124.35	1.35	1.40	1.95	1.81
Males								
Control	16.40	66.20	34.85	117.45	1.57	1.68	1.80	1.73
Experiment	20.95	76.05	38.35	135.35	1.54	1.69	1.79	1.72

Discussion

Studies conducted so far have focussed on the use of glycerol in diets to substitute cereals (Peisker and Dersjant-Li 2006) or maize (Simon et al. 1997). The partial substitution of soybean oil with glycerol is a novel approach of addressing the problem of the energy balance in diets, and it was verified in this study.

The replacement of soybean oil with glycerol resulted in qualitative changes in the composition of diets. Such changes were manifested by increased contents of NNES and NaCl, and decreased contents of total protein, crude fat and starch in experimental diets.

The addition of glycerol had no major effect on the content of individual amino acids and their spectrum. What was considered a beneficial effect from a dietetic point of view was a lowered content of erucic acid. However, the reduction in the contents of essential FAs poses a certain risk. Diets containing 15–20% of maize and soybean oil are sufficiently supplemented with essential FAs even if soybean oil is replaced with glycerol. This statement is also documented by the results of analysis of diets used in our experiment in which the normalized content of linoleic acid was 19.615 $g \cdot kg^{-1}$ (BR 1), 21.291 $g \cdot kg^{-1}$ (BR 2) and 23.265 $g \cdot kg^{-1}$ (BR 3), which exceeds the recommended dose (12.0–12.5 $g \cdot kg^{-1}$ of diet) for broiler chickens during fattening, as reported by Zelenka et al. (2007). From a dietetic point of view, the excessive intake of polyunsaturated fatty acids does not always have a positive effect on chickens' health and is not always beneficial in terms of technological properties of produced meat. The combination of soybean oil and glycerol can be considered a suitable solution.

The replacement of soybean oil with glycerol increased the production efficiency of used diets, which was manifested by an increase in chickens' growth intensity and bodyweight at the end of the experiment on 38 day of age: by 13.22% in females and by 12.12% in males. Simon et al. (1996) or Barteczko and Kaminski (1999) reported a positive effect of glycerol added up to 5% (at a maximum addition up to 10%) into a diet on the performance indicators of broiler chickens. Thacker et al. (1994) did not confirm a positive effect on the performance indicators of chickens with the use of glycerol in a diet. A number of other authors have reported that the content of glycerol exceeding 10% in a diet have a negative effect on the performance indicators in fattened chickens. Although the consumption of diets was higher in experimental groups, feed conversion in experimental groups was better, 1.84 kg (C) and 1.81 kg (G) in females and 1.73 kg (C) and 1.72 kg (G) in males.

There are only few studies regarding the use of glycerol in the nutrition of farm animals. In some species and categories such studies have not yet been conducted. It can be assumed that with the development of rapeseed processing and the increasing production of biodiesel offering large amounts of glycerol as a "waste product", glycerol will become a suitable energy component to be added in feeds for farm animals. One positive finding is the fact that glycerol also improves technological and hygienic quality of granules, as pointed out by Schröder and Südekum (1999). However, a dose of glycerol exceeding 100 $g \cdot kg^{-1}$ of feed may pose some technological problems during granulation.

From a standardization point of view, some problems may arise due to the fact that the composition of crude glycerol generated in the production of methylester varies with the technology of oil processing. Different quality affected by the presence of various impurities can therefore be found in literature sources. Major "impurities" include water, methanol, phosphorus and its compounds, sodium chloride and potassium chloride.

The economic evaluation of the use of glycerol is rather problematic due to varying prices of individual feed components. We assume that the replacement of cereals by glycerol is not suitable since the price of cereals is usually lower than the price of glycerol. However, the situation is different when soybean oil is replaced by glycerol because the price of glycerol is several times lower than the price of soybean oil. The price of glycerol is likely to decrease with the overproduction of glycerol generated in the production of methylester. The use of glycerol in diets will therefore become very interesting from an economic point of view.

It follows from our results and literature data that the optimum amount of glycerol in feed for poultry is 5%. If the diet programme is designed properly, it is possible to use 10% of glycerol in a diet without a negative effect on the performance indicators of animals. However, there are still many issues associated with the use of glycerol in the nutrition of animals that have to be addressed in future research studies.

Glycerol jako energetický zdroj při výkrmu brojlerových kuřat

Cílem práce bylo ověřit možnost náhrady sojového oleje glycerolem v dietě brojlerů a sledovat vliv na užitkovost a zdravotní stav brojlerových kuřat. Do pokusu bylo zařazeno 122 jednodenních kuřat, která byla rozdělena podle pohlaví na dvě kontrolní (30 samic a 31 samců) a dvě pokusné (30 samic a 31 samců) skupiny. U krmných směsí určených pro experimentální skupiny kuřat byla provedena 50% náhrada sojového oleje glycerolem v poměru 1:2. P Kuřata obou pohlaví experimentálních skupin, kterým byly podávány krmné směsi s glycerolem, dosáhla 15., 32. a 38. den výkrmu, statisticky vysoce významně ($p \leq 0.01$) vyšší průměrnou živou hmotnost ve srovnání s kontrolními skupinami. Na konci pokusu měla kuřata kontrolních skupin průměrnou hmotnost 2.078 kg a kuřata experimentálních skupin 2.341 kg. Celková spotřeba krmné směsi za 38 dnů výkrmu byla u samic z kontrolní skupiny 3.588 kg a 4.011 kg u samic z experimentální skupiny, u kohoutků z kontrolní skupiny 3.915 kg a u kohoutků z experimentální skupiny 4.366 kg , tedy vyšší u pokusných kuřat. Konverze krmiva byla u kuřat pokusných skupin lepší, u samic z kontrolní skupiny 1.84 kg a u samic z experimentální skupiny 1.81 kg a u samců z kontrolní skupiny 1.73 kg a u samců z experimentální skupiny 1.72 kg (G2). Na základě našich výsledků lze za optimální množství glycerolu v krmivech pro drůbež považovat 5 %. Práce představuje originální řešení optimalizace krmných směsí náhradou rostlinného oleje glycerolem. Výsledky práce přináší významný produkční i ekonomický dopad ve výkrmu brojlerových kuřat.

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