Quality characteristics of pelleted compound feeds under varying storage conditions as influenced by purity and concentration of glycerol from biodiesel production*

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ABSTRACT

The effects of concentrates, in which glycerol plus soyabean meal replaced wheat, on physical, chemical and hygienic pellet quality characteristics were investigated. Three purities of glycerol were used, reflecting different stages of the biodiesel production process. Glycerol inclusion levels were 50, 100 and 150 g/kg concentrate dry matter (DM). Concentrates were stored under good (15°C, 60% relative humidity) or bad (20°C, 70% relative humidity) environmental conditions and for 4 or 8 weeks. Chemical quality was only slightly affected by purity and concentration of glycerol and likewise by storage conditions or duration. Ergosterol concentration indicated that glycerol of different purity had a preserving effect. The physical quality of pellets was not affected by the purity of glycerol or by glycerol concentration of up to 150 g/kg DM. Glycerol at different purity may help to stabilize the hygienic quality of pelleted compound feeds without compromising the physical quality of pellets.

KEY WORDS: pelleting, pellet quality, glycerol, storage, ergosterol

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INTRODUCTION

The turn towards renewable energy sources in the European Union has increased the production of biofuel from rape seed oil, mainly in the form of rape seed oil methylester (RME) or biodiesel, leaving glycerol as a by-product (Körbitz et al., 2003). If the amount of glycerol from biodiesel production exceeds the needs of pharmaceutical and chemical industries, it could be used in animal nutrition, possibly in the form of less pure and thus cheaper qualities. Glycerol was registered as a feed additive (E 422) in the European Union more than 30 years ago (Anonymous, 1970) with no restrictions as to animal species and quantity added to feeds. Recently, and at least in part as a result of increasing biodiesel production and thus glycerol accumulation, glycerol was listed as a feedstuff in the "Positive List" of authorized feed materials (Normenkommission für Einzelfuttermittel im Zentralausschuss der Deutschen Landwirtschaft, 2007) which, among other objectives, was initiated to contribute to feed safety internationally (Anonymous, 1996) and nationally (Petersen and Flachowsky, 2004).

In past decades, pelleting of feeds has become a firmly established process within the feed industry, because pellets offer numerous advantages compared with meals: improved flow properties in conveying equipment and silos, increased bulk density, dust binding, no segregation of components and reduced feed refusals (Rumpf, 1962). The quality of pellets depends markedly on external factors like storage and technology, as well as on the proportion and physical and chemical properties of raw materials, with a particular role of feed additives like pellet binders or stabilizers (for review, see Thomas and van der Poel, 1996; Thomas et al., 1997, 1998). Due to its hygroscopicity, glycerol can affect the quality of compound feed pellets, because general binding forces of feed particles and water activity in the feedstuffs may be influenced. Therefore glycerol may influence physical, chemical and hygienic variables of feedstuffs. However, it is not clear under which conditions glycerol may result in either improvement or detriment of pellet quality.

The objectives of this study were to evaluate the physical, chemical and hygienic properties of pelleted compound feeds containing glycerol of different purity and at different concentrations. Feeds were stored under different environmental conditions reflecting a range of situations that will also occur in practice.

MATERIAL AND METHODS

Experimental design

The experiment was conducted as a completely randomized design, with a 10×5 factorial (10 concentrates and 5 storage conditions) arrangement of treatments, totalling

50 treatments. Each treatment was replicated 3 times, with a total of 150 experimental units. The ten concentrates resulted from one concentrate without glycerol (Control) and three glycerol concentrations (50, 100 and 150 g/kg dry matter (DM)), each combined with glycerol of three purities (low, medium, high). Environments were defined by an unstored control and two storage conditions, hereafter designated good (15°C, 60% relative humidity) and bad (20°C, 70% relative humidity), each combined with two storage durations, namely four and eight weeks. Three coarsely meshed jute sacks that allowed widely unrestricted air exchange between pellets and ambient air were used for each combination of factors. Sacks were filled with 2 kg pelleted feed.

Purity of glycerol and feed composition

One ingredient of the compound feeds, designated concentrates hereafter, was glycerol of low, medium or high purity (glycerol concentration approximately 600, 800 or 1000 g/kg product), reflecting different stages of the same process of RME production (Ölmühle GesmbH, Bruck/Leitha, Austria). The chemical composition of the three glycerol types is shown in Table 1. Major differences between the glycerols were observed in concentrations of water, glycerol,

Table 1. Chemica	l composition	of glycerol	of different	puritie	2S1
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		Purity of glycerol	
	low	medium	high
Water, g/kg	268.0	11.0	25.0
Dry matter composition,	g/kg unless stated		
glycerol	633.0	853.0	998.0
crude fat	7.1	4.4	NA^2
phosphorus	10.5	23.6	NA
potassium	22.0	23.3	NA
sodium	1.1	0.9	NA
lead, mg/kg	3.0	2.0	NA
methanol	267.0	0.4	NA

¹ samples were analysed in triplicate

phosphorus, and methanol. In order to use some of the concentrates investigated in concurrent animal studies on cattle and sheep (Schröder and Südekum, 1996, 1999), concentrates were formulated on an isonitrogenous and isocaloric (net energy for lactation) basis. Ten concentrates were produced, one batch of each, formulated to contain no glycerol, or 50, 100 or 150 g/kg DM of glycerol from the above mentioned types of glycerol. The ingredient composition of the different concentrates is presented in Table 2.

² NA, not analysed; analyses were omitted because the glycerol content was close to 1000 g/kg and high purity glycerol is listed in the official European Union and United States pharmacopoeias

	C 1				Purity	of glyc	erol			
Ingredient	Control -		low		n	nedium			high	
	0	50	100	150	50	100	150	50	100	150
Wheat, ground	300	230	160	100	230	160	100	230	160	100
Sugar beet pulp, dehydrated	200	200	200	200	200	200	200	200	200	200
Wheat bran	140	140	140	140	140	140	140	140	140	140
Rapeseed meal	120	120	120	120	120	120	120	120	120	120
Soyabean meal	100	120	140	150	120	140	150	120	140	150
Maize	100	100	100	100	100	100	100	100	100	100
Premix ³	40	40	40	40	40	40	40	40	40	40
Glycerol	0	50	100	150	50	100	150	50	100	150

Table 2. Ingredient composition (g/kg dry matter) of pelleted concentrates containing no glycerol (Control) or glycerol of different purities¹ at three different concentrations²

Processing

Concentrates were processed with a 20 kW hammer mill (Wachtel, Reinbek, Germany), equipped with a 3-mm screen, a horizontal batch mixer (ploughshare® mixer FM 130; Lödige, Paderborn, Germany) with a capacity of 90 kg and an 11 kW pellet press (Simon-Heesen, Boxtel, Netherlands). All ingredients except premix and glycerol were ground in a hammer mill, weighed and put into the mixer. Concentrates were mixed in batches of 50 kg. First, the premix was mixed in the horizontal batch mixer, then grains and by-products were added, and finally glycerol was supplemented directly into the mixer. The pellet press consisted of a static die and three rotating rollers. The die size, described by length to diameter ratio (mm:mm) of bore holes, was 40:6. To achieve pellets of the best possible quality, water was added to glycerols of medium and high purity, and the gap-size between roller and die as well as the distance between the pellet die knife and the die face were adjusted as necessary. In order to decrease moisture subsequent to the pelleting process, pellets passed a vibratory cooler (DK-60; Simon-Heesen, Boxtel, Netherlands) at a rate of approximately 2.5 kg/min and a temperature of 30°C.

Pellet quality

The hardness of the pellets was measured using a hardness tester (PTB 301/501; Pharma Test, Hainburg, Germany). The pellets were conditioned overnight at 50°C and randomly chosen to insert them singly between two bars. By increasing static

¹ for details of glycerols, see Table 1

² values are based on dry matter analyses on ingredients and weighed amounts of ingredients before feed mixing

³ mineral vitamin mixture, pellet binder (Diamol®; Franz Bertram, Hamburg, Germany) and titanium(IV)-oxide

pressure applied by means of a spring, the force (newton [N]) needed to crack the pellet was recorded by a force transducer. The average of ten measurements is referred to as the hardness of the pellets.

Durability of pellets was estimated from particle size distribution of a batch of pellets. Fines were induced by an abrasing action of pellets shearing over each other and over the wall and ledges of drums in a tumbling device with four chambers and a circumference of the drum of 138 cm, as described by Scholz (1976). Pellets were conditioned at 30°C overnight; then 500 g of pellets free of abrasion were inserted into each chamber of the drum. A haulage way of 500 m was simulated by 360 rotations of the tumbling can device at 36 rotations per min. Finally pellets were sieved with an electromagnetic sieve shaker (Vibratronic VE-1; Retsch, Haan, Germany) using sieves with square openings of 4.75 and 2.00 mm. The sieve shaker was operated at an amplitude of 0.7 mm for 4 min. Vibrations were interrupted for about 2 sec every 20 sec. The proportion of total sample retained on the 4.75 mm sieve, i.e. $0.8 \times$ pellet diameter, was considered whole pellets; the respective proportion of the sample retained on the sieve with 2.00 mm openings was denoted as broken pellets, and all material that could pass this sieve size was designated as fines.

Sample processing and analytical procedures

Pellets were successively ground in mills with 3 and 1 mm screens and, for starch analysis, with a 0.2 mm screen. The DM of pellets from each jute sack was estimated by oven-drying at 105°C overnight. Ash was determined by ashing in a muffle furnace at 550°C overnight (16 h). Ether extract (petroleum ether extract after acid hydrolysis by the Soxhlet method; method 5.1.1) and crude fibre (method 6.1.4) were analysed according to "Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten" (VDLUFA; Bassler, 1988, 1993). N was determined using a standard Kieldahl procedure using Cu²⁺ as a catalyst (method 4.1.1; Bassler, 1988, 1993) and crude protein (CP) calculated as (N \times 6.25). Starch was quantified after enzymatic hydrolysis of starch to glucose as described by Brandt et al. (1987). Ergosterol was analysed by liquid chromotography (Schwadorf and Müller, 1989) and used as an indicator for fungal biomass. Glycerol determination in pellets started with dispersing 5 g of finely ground (1 mm screen) material or 5 g of one of the three glycerol commodities with 100 ml of double-distilled water in a shaking water-bath at 80°C for 15 min. Then the extract was separated by filtering and glycerol was analysed enzymatically (Glycerol UV Method, catalogue number 148270; Boehringer Mannheim, Mannheim, Germany). The water content of the different glycerol types was determined using an automatic Karl Fischer titrator (Karl Fischer-Titrator E 551; Metrohm, Herisau, Switzerland), dispensing Karl Fischer reagents

(Volumetry-Two-Component Reagents [number 34800 and 34801]; Riedel-de Häen, Seelze, Germany) from a Metrohm Dosimat (E 415, Herisau, Switzerland). The DM content (g/kg) of glycerol was estimated as 1000 minus water. Methanol was analysed using an HPLC method (Siegfried et al., 1984) that is routinely used for monitoring silage fermentation products including alcohols.

Statistical analysis

Data analysis was performed using the general linear models (GLM) procedure of SAS (1988). The model was as follows:

$$\begin{aligned} \textbf{y}_{jk} = \ \mu + concentrate \ type_{j} + environment_{k} + (concentrate \ type \\ \times \ environment)_{jk} + \epsilon_{jk} \end{aligned}$$

where: y_{jk} = observed response, μ = overall mean, concentrate type $_j$ = effect of concentrate type 1 to 10, as outlined in material and methods, environment $_k$ = effect of environment 1 to 5, as outlined in material and methods; (concentrate type × environment) $_{jk}$ = effect of interaction term of concentrate type $_j$ and environment $_k$ and ϵ_{jk} = residual error.

Effects of pellet environment were further separated into linear contrasts. Contrasts were 'unstored vs stored', 'stored 4 weeks vs stored 8 weeks' and 'good vs bad storage conditions'. Further, effects within concentrate types were evaluated by the contrast 'unstored vs stored' (Table 7). Significance was declared at P<0.05 unless otherwise stated. Values are presented as means with their respective SEM.

RESULTS

The chemical composition of the ten unstored concentrates is summarised in Table 3. Because concentrates where formulated to be isocaloric and isonitrogenous, concentrations of starch and glycerol varied, whereas CP concentrations were similar. Actual glycerol concentrations were close to the intended level indicating that preparation of the pellets matched the objectives. Although the methanol concentration of the glycerol of low purity was marked, only negligible quantities of methanol were detected in the pellets (data not shown).

Storage per se and storage duration or conditions did not appear to affect chemical composition of the concentrates and mainly reflected intended differences as observed for unstored concentrates. To quote an example, variation of CP concentration was small and the difference between extreme values was only 15 g/kg DM (data not shown). Therefore, only data for DM are presented hereafter.

Table 3. Chemical composition of unstored concentrates containing no glycerol (Control) or glycerol of different purities1 at three different $concentrations^2$

14.000	1000				Pur	Purity of glycerol	irol			
III	Control		low			medium			high	
				concentra	concentration of glycerol, g/kg dry matter	erol, g/kg d	ry matter			
	0	50	100	150	50	100	150	50	100	150
Dry matter, g/kg	840	698	887	895	854	895	905	854	859	864
Dry matter composition,	n, g/kg									
ash	77	06	26	66	98	88	92	83	82	85
crude protein	191	191	190	187	187	190	184	192	194	192
crude fibre	72	79	79	78	78	82	92	62	81	62
Starch	283	254	218	168	254	226	170	271	223	177
acid ether extract3		24	24	24	25	24	21	25	24	22
glycerol	2	45	93	128	51	96	131	49	101	146
1 for details of almerols	T ees									

¹ for details of glycerols, see Table 1 ² samples were analysed in triplicate

³ acid hydrolysis prior to extraction

Table 4. Effects of storage duration and storage environment on dry matter concentration (g/kg) of pelleted concentrates containing no glycerol (Control) or glycerol of different purities¹ at three different concentrations²

Storage Duration Environment 0			0.000				Purity	Purity of glycerol				
Concentration of glycerol, g/kg dry matter 840 869 887 895 854 895 854 859 854 841 882 871 856 880 869 856 879 862 871 871 887 860 847 873 858 873 885 887 871 887 860 847 873 858 874 844 871 871 870 839 852 859 852 827 864 844 844 4.0			Control		low			medium			high	
nt 0 50 100 150 50 100 840 869 887 895 895 905 854 859 877 882 871 856 880 869 856 879 862 885 887 885 885 885 885 885 885 885 885 885 885 885 885 885 885 887 886 885 885 887 885	S	torage				concentration	n of glycerol	, g/kg dry m	atter			
840 887 895 854 895 854 859 877 882 871 856 880 869 856 879 862 891 897 890 874 899 894 880 893 885 871 887 860 847 873 859 848 858 871 870 839 852 859 848 858 807 4.0 4.001 4.001 4.0001 4.0001 4.0001 4.0001 4.0001	Duration	Environment	0	50	100	150	50	100	150	50	100	150
877 882 871 856 880 869 878 862 891 897 890 874 899 894 880 893 885 871 887 860 847 873 859 848 858 807 871 870 839 852 859 852 827 864 844 4.0 4.0 4.0 4.0 4.0 4.0 4.0 4.0 4.0 4.0 4.0 6.0.001 4.0 6.0.001 4.0 6.0.001 4.0 6.0.001 4.0 6.0.001 4.0 6.0.001 4.0 6.0.001 4.0 6.0	Unstored		840	698	887	895	854	895	905	854	859	864
891 897 890 874 899 894 880 893 885 871 887 860 847 873 859 848 858 807 871 870 839 852 859 852 844 844 4.0 -0.001 -0.001 -0.001 -0.001 -0.001 -0.001 -0.001 -0.001 -0.001 -0.001 -0.001 -0.001	4 weeks	Good	877	882	871	856	880	698	856	879	862	847
871 887 860 847 873 859 848 858 807 871 870 839 852 859 852 844 844 4.0 -0.001 -0.001 -0.001 -0.001 -0.001 -0.001 -0.001 -0.001 -0.001 -0.001 -0.001 -0.001	4 weeks	Bad	891	897	890	874	668	894	880	893	885	867
871 870 839 852 859 852 844 844 4.0 -0.001 -0.001 -0.001 -0.001 -0.001 -0.001 -0.001 -0.001 -0.001 -0.001	8 weeks	Good	871	887	098	847	873	859	848	828	807	793
	8 weeks	Bad	871	870	839	852	859	852	827	864	844	826
	SEM							4.0				
	Effects (P)	Jc										
	storage	(S)						<0.001				
	concent	rate type (C)						<0.001				
	interact	ion $(S \times C)$						<0.001				
	Contrasts fo	ır storage, P										
	unstore	d vs stored						<0.001				
E)	4 weeks	s vs 8 weeks (D)						<0.001				
E)	sv boog	; bad (E)						<0.001				
	interact	ion $(D \times E)$						< 0.001				

¹ for details of glycerols, see Table 1

² each value is the average of three replicates

Table 5. Effects of storage duration and storage environment on hardness (newton [N]) of pelleted concentrates containing no glycerol (Control) or glycerol of different purities1 at three different concentrations2

Storage Control Storage Duration Environment E							Puri	Purity of glycerol	rol			
Concentration of glycerol, g/kg dry matter			Control		low			medium			high	
nent 0 50 100 150 50 100 150 50 100 150 50 100		Storage				concer	ntration of g	lycerol, g/l	kg dry matte	L		
220 227 218 331 196 207 211 251 140 241 236 236 339 178 260 242 205 158 237 246 284 428 191 195 195 258 148 238 181 250 327 206 238 234 187 210 237 173 324 396 256 194 253 194 156 19 257 173 324 396 256 194 253 194 156 19 257 173 324 396 256 194 253 194 156 19 258 188 210 237 246 284 428 191 195 195 210 238 181 250 238 234 187 210 240.001 250 2	Duration	Environment	0	50	100	150	50	100	150	20	100	150
241 236 236 339 178 260 242 205 158 237 246 284 428 191 195 195 258 148 238 181 250 327 206 238 234 187 210 237 173 324 396 256 194 253 194 156 19	Unstored		220	227	218	331	196	207	211	251	140	127
237 246 284 428 191 195 195 258 148 238 181 250 327 206 238 234 187 210 237 173 324 396 256 194 253 194 156 19 60.083 <0.001 <0.045 0.095 0.095 0.095	4 weeks	Good	241	236	236	339	178	260	242	205	158	68
238 181 250 327 206 238 234 187 210 237 173 324 396 256 194 253 194 156 19 6.083 <0.001 <0.045 0.045 0.085	4 weeks	Bad	237	246	284	428	191	195	195	258	148	120
237 173 324 396 256 194 253 194 156 19 0.083 												

1 for details of glycerols, see Table 1

² each value is the average of three replicates

Table 6. Effects of storage duration and storage environment on proportion (g/kg concentrate) of whole pellets1 past abrasing procedure of concentrates containing no glycerol (Control) or glycerol of different purities² at three different concentrations³

		London				Pur	Purity of glycerol	erol			
		Control		low		medium	ium			high	
9 1	Storage			3	oncentrati	concentration of glycerol, g/kg dry matter	rol, g/kg c	lry matter			
Duration	Environment	0	50	100	150	50	100	150	50	100	150
Unstored		771	843	973	936	847	947	656	962	824	875
4 weeks	Good	795	879	211	961	885	948	926	892	853	688
4 weeks	Bad	819	860	1000	943	910	971	955	818	855	885
8 weeks	Good	786	884	975	396	884	976	211	733	466	890
8 weeks	Bad	831	861	666	942	893	826	949	824	851	888
SEM							12.0				
Effects (P) of											
storage (S)							<0.00	1			
concentrate	type (C)						<0.001	1			
interaction (S \times C)	$(S \times C)$						<0.00	1			
Contrasts for sto	orage, P										
unstored vs stored	stored						<0.00	1			
4 weeks vs	4 weeks vs 8 weeks (D)						0.12	4			
good vs bad (E)	d (E)						<0.001	1			
interaction $(D \times E)$	$(D \times E)$						0.161	1			
20 1 7 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	2	1-T T-1-1	1 3 2 2 2	1 1 1	4	f. 41	2012.01				

particles ≥ 4.75 mm, ² for details of glycerols, see Table 1, ³ each value is the average of three replicates

The DM concentration of unstored concentrates varied from 840 g/kg for the control to 905 g/kg for the concentrate with 150 g/kg DM medium-purity glycerol (Table 4). Within glycerol purity, DM increased with increasing concentration of glycerol. Unlike unstored concentrates, the stored concentrates contained less DM, or more moisture, with increasing concentrations of glycerol. Significant effects were observed of storage and concentrate type and their interaction. The contrasts for storage revealed differences (P<0.001) between unstored and stored concentrates, between 4 and 8 weeks of storage, and between good and bad environments. Longer storage and bad storage conditions reduced DM and thus increased moisture contents of the pellets.

Unstored concentrates had 2.9 to 3.4 mg/kg DM of ergosterol (data not shown). Only after the concentrate without glycerol (Control) had been stored for 8 weeks, a sharp increase of ergosterol (11.4 mg/kg DM) concentration was detected. This value was considerably higher than in all other stored materials, which ranged only from 1.7 to 3.4 mg/kg DM of ergosterol, thus resembling values of unstored pellets.

Hardness of unstored concentrates ranged from 127 to 331 N. The lowest values were observed for concentrates with 100 and 150 g/kg DM of high-purity glycerol, pellets of the control had medium hardness and the greatest values were associated with the pellets containing 150 g/kg DM low-purity glycerol (Table 5). This ranking was the same for all stored concentrates regardless of storage duration and condition. Effect of concentrate type on hardness was significant (P<0.001) and approached significance (P=0.083) for an effect of storage environment. As stated by contrasts for storage, duration (4 weeks vs 8 weeks) did not cause differences, whereas storage per se (unstored vs stored) led to an increase of pellet hardness. No interactions were observed between storage duration and conditions.

Particle size distribution of pelleted concentrates as indicated by the proportion of whole pellets is presented in Table 6. The unstored concentrates had 771 to 973 g/kg of whole pellets. The lowest value was recorded for the control, and the highest value for the pellets with 100 g/kg DM of low-purity glycerol. Effects (P<0.001) were observed on proportion of whole pellets of storage and concentrate type as well as an interaction between these effects. The contrasts for storage showed marked effects (P<0.001) of storage per se (unstored vs stored) and of storage conditions (good vs bad). Change of proportion of whole pellets inevitably led to a change of proportion of other fractions, namely broken pellets and fines (data not shown).

The comparison of unstored and stored products within concentrate type, as presented in Table 7 clearly shows the effects of concentrate type on DM contents and physical pellet quality. DM contents of all types of concentrates were significantly altered by storage. While particle size distribution of pellets without glycerol (Control), with 50 and 100 g/kg DM of glycerol, and of types with low and medium purity was affected by storage, no effects were observed for concentrate types with either 150 g/kg DM glycerol or high purity glycerol. These effects were

related to hardness of pellets. However, the most pronounced effects on hardness were found for concentrate types with glycerol at either 100 g/kg DM or of low purity at all concentrations.

Table 7. Contrasts (P values) for unstored versus stored concentrates within concentrate type for dry matter concentration (g/kg) and physical pellet properties

			(Concentrate	type		
Item	concen		glycerol, atter	g/kg dry	Pur	rity of glycer	ol¹
	0	50	100	150	low	medium	high
Dry matter	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.013
Hardness	0.386	0.211	0.007	0.093	0.028	0.192	0.730
Particle size distribution ²							
whole pellets	0.007	0.007	0.080	0.149	0.011	0.011	0.439

¹ for details of glycerols, see Table 1, ² whole pellets: ≥ 4.75 mm

DISCUSSION

The magnitude of the changes of chemical composition caused by storage duration and condition was always small. These observations upon effects of storage on chemical composition of the pelleted concentrates are in line with findings on the hygienic quality of the pellets. Significant modifications of chemical composition during storage would have indicated enhanced microbial activity and growth, paralleled by an increase in microbial and, particularly fungal, biomass for which ergosterol has been used as a chemical marker in cereal grains and grain by-products (Müller and Lehn, 1988; Müller et al., 1994) and compound feeds (Bailly et al., 1999). The vast majority of ergosterol concentration was considerably lower than 'preliminary normal ranges' for these types of feeds (6.1 to 6.6 mg/kg; calculated according to Müller and Schwadorf, 1988, 1990), therefore indicating products of excellent hygienic quality. Only eight weeks of storage of the control concentrate under bad conditions increased ergosterol concentration drastically (11.4 mg/kg), but still chemical composition was only modified slightly. Thus, storage longer than eight weeks or more sensitive analytical measures seem to be necessary to monitor chemical changes due to fungal activity and growth on the types of feeds studied here. It should not be overlooked that elevated ergosterol concentration is also a warning sign of possible mycotoxin contamination by moulds (Müller and Schwadorf, 1988).

In summary, it can be ascertained that glycerol of different purity and even at relatively low concentration (50 g/kg DM) had a preserving effect, likely caused

by the hygroscopicity of glycerol, which decreases water activity and thus, water availability to (undesirable) microorganisms in the pellets (Heiss and Eichner, 1994). This preserving effect of glycerol is widely used in the food industry (Baltes, 1995). An interesting aspect of glycerol's impact on microbial activity was reported by Roger et al. (1992). These authors found that *in vitro*, glycerol at a concentration of 50 g/kg in the incubation medium totally inhibited the cellulolytic activity of a strictly anaerobic rumen fungal species, *Neocallimastix frontalis*. This effect was probably caused in part by the inhibition of its growth, but the authors also discussed a possible effect on fungal cellulases.

The pellets investigated here were manufactured under conditions where process variables during pelleting could not be generally adjusted, because both concentration and purity of glycerol had an impact on the pellet production process. In order to produce unstored pellets of the best possible similarity in physical quality variables, it was necessary to adjust the pelleting process intuitively according to visual pellet appraisal by an experienced operator.

Hardness is an important quality characteristic for resistance of pellets to compressive forces during handling and storage. Values for durability and hardness are strongly dependent on devices and conditions used for their determination (Thomas and van der Poel, 1996). Therefore, only results derived from the same device can be reliably compared. No values have been reported so far using the same hardness assessment procedure as in this study, probably because this equipment is used more frequently in the pharmaceutical industry to evaluate tablet hardness. Moreover and unfortunately, abrasion devices in the feed industry are not internationally standardized and several types of equipment are available. Nevertheless, evaluation and comparison of the hardness and durability of concentrates of the present study should allow a relative ranking of treatments as related to different concentrations and purities of glycerol and to different storage durations and conditions.

The mechanisms of binding during pelleting are based upon natural adhesion between particles and the mechanical load which forces inter-particle contact. As already formulated almost 50 years ago by Rumpf (1958) and fully developed by Friedrich (1964) and Friedrich and Robohm (1968), various binding phenomena occur (recent reviews by Thomas and van der Poel, 1996 and Tabil et al., 1997): a. solid-solid interactions; b. capillary pressure and interfacial forces resulting in so-called 'liquid neckings'; c. adhesive and cohesive forces between particles and binders; d. interactions between particles due to folding and plying, and e. form-closed bonds or interlocking occurring in fibres, flattened or bulky particles. In our study, the most causative factors for expected differences of physical pellet quality variables would be 'stick' and hygroscopicity of glycerol, resulting in immobile liquid neckings, as well as mechanical interlocking, possibly also impacted by chemical composition of the concentrates. The latter force, caused by pressure

applied during pelleting, could delay redistributing of binding agents around the particles and the structural integrity of the pellets may change with storage duration (Pfost and Young, 1973). Related to time that is taken for redistribution of glycerol through the pellets, time related migration of water as caused by hygroscopic properties of the agent would happen and forming of liquid neckings in the inner pellet and thus hardness should increase. This is in line with our results: storage alone (unstored vs stored) led to an increase in hardness of the pellets, whereas storage duration (4 vs 8 weeks) did not affect hardness. This may indicate that redistribution of binding agents, or at least moisture adsorption, is not finished after cooling and before placing the pellets in storage. It is to be supposed that the lack of response of extension of storage duration on hardness is attributed to completion of redistribution of the binding agent and moisture adsorption after less than 4 weeks of storage. However, moisture equilibrium obviously was not reached within a 4-week period, because the moisture of all treatments, except the control, increased from 4 to 8 weeks of storage.

When hardness increased with decreasing pellet moisture concentrations at increasing proportions of glycerol (Table 4: unstored concentrates; glycerol of low and medium purity), total binding forces could have decreased due to a smaller amount of liquid necking. However, force per bond would then increase since water in the larger capillaries is evaporated first (Thomas and van der Poel, 1996). Subsequently, the remaining water would have established bonds between smaller particles, resulting in moister pellets being harder, as found for concentrates with glycerol of low and medium purity. On the other hand, no positive response to hardness on increasing concentrations of high-purity glycerol was observed. Hygroscopicity of glycerol should first have resulted in increased moisture concentration. However, the decrease of hardness of pellets with increasing concentration of high-purity glycerol appears to be related to moisture concentration rather than being of uniform magnitude. Despite the decrease of hardness, the proportions of fines were still similar to or even lower than those of the control, adding further evidence that glycerol positively affected physical pellet quality. When glycerol, containing 200 g/kg water, which was very similar to the medium purity of glycerol in our study, was used for manufacturing high-fat broiler pellets and molassed pellets for fattening bulls at glycerol concentrations of 0, 30, 50, 80 and 100 g/kg, abrasion, i.e. the proportion of fines of total pellet mass, also decreased with increasing glycerol proportion (Löwe, 1999). When pellets were produced with molasses, however, glycerol at concentration greater than 50 g/kg resulted in a rough and scaly surface (Löwe, 1999). This author also noted that when feeds are stored in meal form, glycerol concentration greater than 50 g/kg may result in lump formation, and therefore suggested restricting the glycerol concentration in pelleted compound feeds to 60-70 g/kg based on general storage behaviour including storage in large silos.

For the majority of the stored concentrate types, hardness increased with increasing concentration of glycerol, except for the high-purity glycerol, which was already considered above. It is obvious that extent of variation of hardness is more marked for glycerol of low quality. This may be caused by the main differences between the glycerols of different purity: concentrations of water and glycerol (Table 1) differed markedly, leading to different water contents before pelleting. Although water was added to glycerols of medium and high purity and the pelleting process was adjusted intuitively based on judgement and experience of the operator, different purities of glycerol may have affected the pelleting process differently and as a result, the physical structure of the pellets might have been modified. However, differences in the pelleting process due to differences in physical pellet variables but also, differences in initial moisture - after pelleting and cooling but before storage - could have exerted an effect due to the hygroscopicity of glycerol.

CONCLUSIONS

The concentrate without glycerol (Control) had the poorest properties in terms of durability and displayed only medium hardness values, indicating a positive effect of glycerol per se. The ability of glycerol to counteract development of undesired microbes, particularly fungi, without negatively affecting physical pellet quality, might be of particular importance for its further use. The results of this study using a limited number of quality criteria suggest that glycerol of different purity can be safely used in pelleted compound feeds in proportions up to 150 g/kg DM. Studies using a broader range of methods and an economic assessment will expand the basis for decision-making as to the use of glycerol as a compound feed ingredient. The present data provides further support to the use of glycerol as a versatile feedstuff.

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