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Effect of deep-stacking broiler litter on pathogenic bacteria, intake, digestibility, microbial protein supply and rumen parameters in sheep

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ABSTRACT

To assess the effectiveness of the deep-stacking process on pathogenic bacteria in broiler litter (BL), the raw BL was deep-stacked in 1 × 1 × 1.5 m plastic bins with 250 or 350 g moisture/kg at the depths of 30, 60 and 120 cm for a period of 21 days. The chemical composition and microbial populations in raw and deep-stacked BL (DBL) were determined. The effect of feeding DBL on nutrient intakes, digestibility, microbial protein supply (MPS) and rumen parameters was assessed using different levels (0, 70, 140 or 210 g/kg diet dry matter [DM]) of DBL with 350 g moisture/kg in the diet of male Moghani sheep (four sheep per treatment), in a 28-day experiment. The deep stacking process of BL increased the contents of ash and ash-free neutral detergent fibre (NDFom), and decreased the crude protein (CP) and non-fibre carbohydrates (NFC) (P<0.05). Increasing moisture content resulted in increase of ash and reduction of NFC concentration in DBL (P<0.001). Compared to the raw BL, the populations of total bacteria, *Escherichia coli*, and other *Coliforms* in DBL were decreased (P<0.05), with the exception of depth 120 cm at 250 g moisture/kg. No *Salmonella* population was observed at depths 30 and 60 cm with 250 and 350 g moistures/kg, and at depth 120 cm with 350 g moisture/kg (P<0.05). Dietary inclusion of DBL resulted in linear increase of the DM, organic matter (OM) and CP intakes and quadratic increases of the NDFom and ash-free acid detergent fibre intakes (P<0.05). The digestibility coefficients of DM, NDFom and CP were increased linearly (P<0.05), and OM digestibility increased quadratically (P=0.036) as the dietary level of DBL was raised. The MPS increased quadratically (P=0.011) with dietary inclusion of DBL. Adding DBL to the diet linearly decreased (P<0.05) the ruminal ammonia-N concentration and acetate to propionate ratio, and linearly increased (P<0.05) the total volatile fatty acids. The counts of total protozoa and sub-family *Entoniniinae* were decreased linearly (P<0.05) as the level of DBL in the diet was raised. Overall, to obtain a safe feedstuff, BL should be deep stacked at 350 g moisture/kg, as this led to a decrease in pathogenic bacteria numbers to a safe level for ruminant consumption. Compared to the control group, feeding sheep with DBL containing 350 g moisture/kg, up to 210 g/kg diet DM, improved feed intake, digestibility, MPS and ruminal fermentation, without any adverse effects on animal health. However, the maximum animal response was observed in the sheep fed 140 g DBL/kg diet DM.

Abbreviations: ADFom, ash-free acid detergent fibre; CP, crude protein; DBL, deep-stacked broiler litter; DM, dry matter; DMD, *in vivo* DM digestibility; Lignin(sa), lignin measured by solubilisation of cellulose with sulphuric acid; ME, metabolisable energy; MPS, microbial protein supply; NDFom, ash-free neutral detergent fibre; OM, organic matter; PD, purine derivatives; TPD, total purine derivatives; VFA, volatile fatty acids; NFC, non-fibre carbohydrates.

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1. Introduction

Using agro-industrial by-products, such as broiler litter (BL) may be a useful way to overcome the shortage of animal feedstuffs in many countries. In Iran, the production of this by-product amounts to approximately 1.5 million t/year (Statistical Centre of Iran, 2013). Utilization of BL as a feedstuff for sheep is beneficial due to the high level of crude protein (CP) (238 g/kg dry matter (DM); Azizi-Shotorkhoft et al., 2012), minerals and digestible DM (650–680 g/kg DM) (Mavimbela and Van Ryssen, 2001). The use of BL in ruminant feeding led to decreased production costs and a higher total production, and is a means of disposing of a waste by an environmentally friendly method (Elemam et al., 2009). However, owing to the presence of pathogenic bacteria it should be processed before offering to ruminants (McCaskey and Anthony, 1979). Thermally processed BL using indirect vapour pressure at 80 °C for 20 min has been successfully used to kill pathogenic bacteria (Azizi-Shotorkhoft et al., 2012). However, transferring BL from broiler house to processing facility could increase the risk of spreading pathogens from one area to another. Therefore, deep-stacking in the broiler house may be a safer and also a cheaper method of producing safe BL before moving it to a sheep farm. Chaudhry et al. (1996) claimed that the deep stacking of BL is feasible and effective in the elimination of pathogens. Deep-stacking BL for few days led to an increase in the temperature up to 60 °C in the stack which was enough to kill pathogens (Chaudhry et al., 1998). Several researchers investigated the potential use of deep-stacked BL (DBL) in ruminant's feeding. The DBL could be incorporated into the diet of ruminants up to 500 g/kg without any adverse effect on animal health (Chaudhry et al., 1996). In another study, the inclusion of DBL (deep stacked in underground silo pit), at up to 450 g/kg of concentrate in the diet did not impact negatively on lamb's performance and health (Elemam et al., 2009). The BL contains Cu which has an inhibitory effect on the activity of ruminal protozoa (Kišidayová et al., 2000). Recently, Vardyova et al. (2006) reported that when sheep were exposed to a prolonged intake of Cu-containing pasture, the total population of rumen ciliate protozoa was significantly reduced. The combined effect of different depths and moisture levels on the pathogenic bacteria population in DBL was not reported. Moreover, there are no data available in the literature on the effect of DBL on protozoa numbers and microbial protein supply (MPS) in the rumen.

This experiment, therefore, was carried out to investigate the simultaneous effect of different depths of stacking at two moisture levels and their interaction on pathogenic bacteria populations in DBL, and to assess the effect of feeding different levels of DBL on the intake, digestibility, MPS and rumen parameters in sheep.

2. Materials and methods

2.1. Deep-stacking of broiler litter

The BL was obtained from the broiler house located in the Animal Science Research Institute (Karaj, Iran) in December (2012). The BL was divided into two equal quantities using a digital scale. After spreading each section of BL on separate plastic sheets, sufficient amounts of water were added to each batch of litter to bring their moisture contents to 250 or 350 g/kg, respectively. Then, the litters having moisture levels of 250 or 350 g/kg were stacked in separate $1 \times 1 \times 1.5$ m plastic bins with no cover for 21 days (three replicates). The stacks were not covered.

Temperature was measured daily using digital thermometers placed at different depths (*i.e.*, 30, 60 and 120 cm) from the surface of the DBL. When the maximum temperature of DBL at the different depths in each bin was reached, the representative samples were taken for later bacterial analysis.

2.2. Biological analysis

Concentration of total bacteria (total plate count) was determined by pour plate technique on plate count agar (PCA), counting the colonies developed after incubation at 37 °C for 24 h (APHA, 1998). *Salmonella* in the samples was determined using Xylose lysine deoxycholate agar (XLD agar) medium (Collee et al., 1996). The counts of *Escherichia coli* and other *Coliforms* were determined using MacConkey agar medium (Holt, 1994). The media were transferred to sterile plates after sterilization (autoclaved at 120 °C and 1.5 atm for 15 min). One gram of each fresh litter sample (on DM basis) was added into 9 mL of buffered peptone water, mixed well and a dilution series was prepared using buffered peptone water. Then 20 μL of each dilution was spread onto the medium, with three replicates (plates) and two observations in each. The plates were incubated for 24 h at 37 °C. At the end of incubation time, the bacterial colonies were counted on the plates.

2.3. Animal study

Sixteen male Moghani sheep with average live weight 62 ± 2.3 kg were housed individually in metabolism crates to allow total collection of faeces and urine. The animals were used in a completely randomized design with four sheep per each diet-treatment.

The complete removal of pathogens was noted with the BL that had been deep stacked with 350 g moisture/kg at 30 and 60 cm depths (Table 2), so an equal mixture of these DBLs was used in the animal study in the present experiment. Four diets (Table 3) containing different levels (0, 70, 140 or 210 g/kg DM of diet) of DBL (air dried) were formulated according to NRC (2007). Each diet was offered *ad libitum* twice daily and fresh water was available at all times. During the experiment,

feed distributed to each sheep and the corresponding residual were recorded daily. Representative samples of feed and residual were bulked for later analyses. To estimate voluntary intake for each sheep, the nutrient content in refused feed was subtracted from that offered in feed.

The digestibility study lasted for 28 days with a 21 days adaptation period to the metabolism crates and diets, and 7 days for sample collection according to Givens et al. (2000). In the last week of the experiment, samples of feeds and faeces from each sheep on each treatment were weighed and 10% representative samples were frozen until analyses. The digestible organic matter (OM) in DM (*D*-value) was calculated using the following equation:

$$D\text{-value}\,(\text{g/kg DM}) = \frac{[\text{OM intake}\,(\text{g}) - \text{faecal OM}\,(\text{g})]}{\text{DM intake}\,(\text{g})}.$$

The metabolisable energy (ME) content for each diet was estimated using the following equation (AFRC, 1993):

$$\text{ME}\,(\text{MJ/kg DM}) = 0.0157 \times D\text{-value}\,(\text{g/kg DM}).$$

At the same time, urine samples from each sheep during 7 days were measured individually and collected in a bucket containing 100 mL of sulphuric acid solution (100 mL/L), placed below the urine outlet in the metabolism crates. Sub-samples of 20% were diluted threefold and then stored at −20 °C for later analysis.

2.4. Rumen fluid sample

The rumen fluid samples were collected by stomach tube from all the animals on day 7 of the collection period, just before morning feeding and 3 h after feeding, and samples were strained through two layers of cheesecloth.

The pH value was measured immediately after sampling and 5 mL of rumen fluid was added to 1 mL of HCl 0.2N, and frozen (−20 °C) for ammonia-N analysis.

For the analysis of volatile fatty acids (VFA), 1 mL of rumen fluid was added to 0.25 mL of an acid solution containing 200 mL/L of orthophosphoric acid and 20 mM 2-ethyl-butyric acid and then frozen at −20 °C.

For rumen ciliates, 2 mL of rumen fluid was pipetted into screw-capped test tubes containing 5 mL of formalinized physiological saline (20 mL formaldehyde in 100 mL saline containing 0.85 g sodium chloride in 100 mL distilled water). After that, two drops of brilliant green dye (2 g brilliant green and 2 mL glacial acetic diluted to 100 mL with distilled water) were added to the test tube, mixed thoroughly and allowed to stand overnight at room temperature. Total and differential counts of protozoa were made in 30 microscopic fields at a magnification of ×20 in a haemocytometer (Neubauer improved, Marienfeld, Germany).

2.5. Laboratory analysis

Samples of DBL, diets, and faeces were dried at 60 °C until a constant weight, ground to pass through 1 mm sieve and stored in plastic containers for chemical analyses. Dry matter, CP and ash were analysed according to the methods described by AOAC (1990). The determination of ash-free neutral detergent fibre (NDFom) was performed without sodium sulphite and expressed exclusive of residual ash according to Van Soest et al. (1991). Ash-free acid detergent fibre (ADFom) was determined and expressed exclusive of residual ash (AOAC, 1990; #973.18). Lignin(sa) was measured by solubilisation of cellulose with 720 g/kg sulphuric acid (Robertson and Van Soest, 1981). The ME content of DBL was estimated by the following equation (Deshck et al., 1998):

$$\text{ME}\,(\text{MJ/kg DM}) = \text{digestible OM of DBL}\,(\text{g/g DM}) \times 18.5\,(\text{MJ/kg DOM}) \times 0.80$$

Rumen ammonia nitrogen was determined according to Broderick and Kang (1980). For VFA analysis, strained rumen fluid samples were centrifuged (14,000 rpm, 5 °C, 15 min) and the concentrations of individual VFAs were determined by the gas chromatography procedure.

Urinary purine derivatives (PD) were determined by spectrophotometric method, as described by Chen and Gomes (1992). Allantoin was measured in urine by colorimetric method at 522 nm after its conversion to phenyl hydrazone. The sum of xanthine and hypoxanthine was calculated by their conversion to uric acid with xanthine oxidase (Sigma; Catalogno. X-1875, 5 Units, Germany), with sub-sequentoptical density at 293 nm. The uric acid was measured from the reduction in optical density at 293 nm following degradation of uric acid to allantoin with uricase (Sigma; Product no. U-9375, Germany). Based on Chen and Gomes (1992) technique, the non-linear equation for describing the quantitative relationship between absorption of microbial purines and excretion of PD in urine is: $Y = 0.84X + (0.150W^{0.75}e^{0.25x})$ where Y is the daily urinary PD excretion in mmol/d, X is the daily absorbed exogenous purines in mmol/d, and $W^{0.75}$ is the metabolic body weight (kg) of the animal. The calculation of X from Y based on the above equation can be performed by means of the Newton–Raphson iteration process as given below:

$$X(n+1) = Xn - \left[\frac{f(Xn)}{f(Xn)}\right]$$

where $f(X) = 0.84X + 0.150W^{0.75}e^{-0.25X} - Y$ and the derivatives of $f(X) = (0.84 - 0.038W^{0.75}e^{-0.25x})$.

Table 1
Chemical composition (g/kg DM or as stated) of deep-stacked broiler litter (DBL).

Items	CP	EE	Ash	NDFom	ADFom	Lignin (sa)	Copper (mg/kg DM)	NFC
Before deep-stacking	255[a]	36.1[a]	151[c]	380[b]	187[b]	85.0	57.6	178[a]
After deep-stacking								
D30 × M250	233[b]	33.7[ab]	176[b]	406[a]	192[b]	86.0	56.6	151[b]
D60 × M250	236[b]	33.1[b]	175[b]	403[a]	189[b]	86.5	58.3	153[b]
D120 × M250	231[b]	32.0[b]	173[b]	410[a]	198[ab]	88.5	56.8	154[b]
D30 × M350	238[b]	32.5[b]	191[a]	418[a]	202[ab]	91.0	58.4	121[c]
D60 × M350	239[b]	32.1[b]	191[a]	413[a]	225[a]	90.3	57.8	125[c]
D120 × M350	239[b]	32.1[b]	189[a]	410[a]	202[ab]	90.0	58.1	130[c]
SEM	1.93	0.78	1.29	4.49	8.01	4.24	1.29	5.23
P-value								
M	0.07	0.36	<0.001	0.17	0.44	0.14	0.96	<0.001
D	0.45	0.41	0.21	0.50	0.69	0.94	0.89	0.44
M × *D*	0.55	0.64	0.91	0.53	0.18	0.76	0.79	0.84

CP, crude protein; EE, ether extract; NDFom, ash-free neutral detergent fibre; ADFom, ash free acid detergent fibre; Lignin(sa), lignin determined by solubilisation of cellulose with sulphuric acid; NFC, non-fibre carbohydrates.

Finally, the produced microbial nitrogen was estimated by the following equation:

$$\text{Microbial N}\,(\text{g/d}) = \frac{[X\,(\text{mmol/d}) \times 70]}{[0.116 \times 0.83 \times 1000]} = 0.727X$$

2.6. Statistical analysis

Data on the temperature of deep-stacked broiler litter were analysed as repeated measurements using the MIXED procedure of SAS (SAS Institute, Version 8.2, Cary, NC, USA), with treatment, and sampling day (continuous variable), and their interactions as fixed effects. The compound symmetry was used as a covariance structure in the model. Least squares means were separated using the DIFF option.

Data on the effect of DBL on pathogenic bacteria were analysed using the GLM procedure of SAS. A randomized complete block design with a split plot arrangement of the treatments, three replications for each treatment, was used to determine the effect of DBL on pathogenic bacteria. Main plot factors consisted of two moisture levels (250 and 350 g/kg litter) and subplot factors included three depths of stack (30, 60 and 120 cm). For the effect of DBL on pathogenic bacteria, the means were compared using Duncan's Multiple Range Test.

The effect of feeding DBL on digestibility, microbial protein supply (MPS) and rumen parameters was analysed using a completely randomized design. A polynomial contrast was used to test the linear or quadratic effects of DBL on measured traits.

3. Results

3.1. Chemical composition

Deep stacking process of BL increased (P<0.05) the contents of ash and NDFom, and decreased CP and non-fibre carbohydrates (NFC) (Table 1). Increasing moisture content resulted in increase of ash and reduction of NFC concentration in DBL (P<0.001).

3.2. Temperature of DBL

For all the treatments, the temperature of DBL increased rapidly and reached a maximum between 9 and 12 days (Fig. 1). Maximum temperature was lower at depth 120 cm than depths 30 and 60 cm. The temperature of DBL at depths 30 and 60 cm reached its maximum level faster than at depth 120 cm. On day 9, the maximum temperatures for depth 30 cm with 250 and 350 g moistures/kg were 56.2 and 60.2 °C, respectively. These values for depth 60 cm with moistures of 250 and 350 g/kg were 54.0 and 56.4 °C, respectively. The maximum temperatures for depth 120 cm with moistures 250 and 350 g/kg on day 12 were 47 and 51.7 °C, respectively. Moisture and depth × moisture interaction affected (P<0.05) the temperature of DBL.

The mean ambient temperature during the current study was 8 °C. On day 21, the minimum temperature of 28.9 °C was observed for DBL containing 250 g moisture/kg and depth 60 cm, which was about 20 °C higher than ambient temperature.

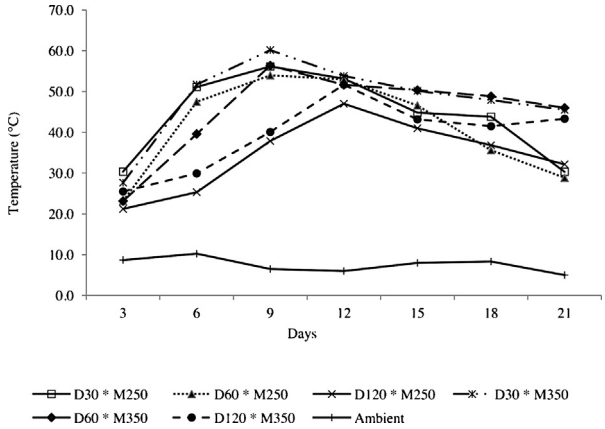

Fig. 1. Effects of different depths and moistures on temperature of deep-stacked broiler litter (DBL).

3.3. Biological analysis

The total bacteria population in DBL decreased (P<0.05) compared to the raw BL, with the exception of depth 120 cm at 250 g moisture/kg (Table 2). No *Salmonella* were observed at depths of 30 and 60 cm with 250 and 350 g moistures/kg, or at depth 120 cm with moisture 350 g/kg (P<0.05). The DBL contained 350 g moisture/kg was free of *E. coli*. There were no colonies of *Coliforms* detected at depth 30 cm with 250 and 350 g moisture/kg, and at depth 60 cm with 350 g moisture/kg. The effect of depth and depth × moisture interaction on populations of *E. coli* and other *Coliforms* was significant (P<0.05). In other words, increasing the depth of the stack increased *Coliforms* in DBL with moistures of 250 and 350 g/kg, *E. coli* in DBL with moisture level at 250 g/kg, and also resulted in *Salmonella* detection in DBL at moisture level 250 g/kg. The populations of total bacteria, *E. coli* and other *Coliforms* were affected (P<0.05) by the level of moisture. Increasing the moisture level of DBL at all the depths decreased the populations of *E. coli* and other *Coliforms*.

3.4. Nutrient intake

The DM, OM and CP intakes increased linearly with increasing dietary DBL. The intakes of NDFom and ADFom increased quadratically (P<0.05) as the DBL incorporated in the diet increased (Table 4). Among the intake of nutrients, the minimum significance of quadratic effect of DBL rate was P=0.013 for ADFom.

3.5. In vivo apparent digestibility of nutrients

The digestibility coefficients of DM (P=0.006), OM (P<0.04), NDFom (P=0.006) and CP (P=0.030) increased with the inclusion of DBL in diet (Table 5). There was a quadratic response of digestibility to increasing levels of DBL in the diet, so that

Table 2
Effect of different depths and moistures on average temperature and survival of pathogenic bacteria in deep-stacked broiler litter (DBL).

Items	Average temperature	Log_{10} cfu/g			
		Total bacteria	*Salmonella*	*E. coli*	Other *Coliforms*
Before deep-stacking		10.6^{a}	2.67	7.43^{a}	6.56^{a}
After deep-stacking					
D30 × M250	44.3^{b}	8.83^{bcd}	ND	1.50^{b}	ND^{b}
D60 × M250	41.2^{c}	9.17^{bc}	ND	1.30^{b}	1.70^{b}
D120 × M250	34.5^{e}	9.73^{ab}	1.53	5.60^{a}	6.30^{a}
D30 × M350	48.1^{a}	8.50^{cd}	ND	ND^{b}	ND^{b}
D60 × M350	45.0^{b}	8.03^{d}	ND	ND^{b}	ND^{b}
D120 × M350	38.4^{d}	8.43^{cd}	ND	ND^{b}	1.27^{b}
SEM	0.446	0.316	1.156	0.810	0.966
P-value					
M	<0.001	0.35	0.39	0.041	0.003
D	<0.001	0.006	0.34	0.002	0.009
Day	<0.001				
M × *D*	<0.001	0.33	0.39	0.041	0.043
M × *D* × Days	<0.001				

Log_{10} cfu/g, colony forming unit per gram of litter sample; *D*, depth (cm); *M*, moisture (g/kg); SEM, standard error of means.
Means with different superscripts in the same column are different (P<0.05).

Table 3
Ingredients and chemical composition (g/kg or as stated) of the experimental diets contained deep-stacked broiler litter (DBL).

Items	Level of DBL in diet (g/kg DM)			
	0	70	140	210
Ingredients				
Alfalfa hay	650	605	559	514
Wheat straw	350	325	301	276
Deep-stacked BL	0	70	140	210
Chemical composition				
DM (g/kg fresh weight)	937	936	935	933
CP	102	112	122	132
NPN (g/kg total N)	213	229	244	260
NDFom	517	508	499	490
ADFom	376	364	352	340
Lignin(sa)	85.1	86.3	87.7	89.0
Ash	92.0	99.3	106	113
Copper (mg/kg DM)	7.95	11.5	15.0	18.5
ME (MJ/kg DM)	7.64	7.77	7.89	8.01

DM, dry matter; CP, crude protein; NPN, non-protein nitrogen; NDFom, ash-free neutral detergent fibre; ADFom, ash-free acid detergent fibre; ME, metabolisable energy.

Table 4
Effect of the experimental diets on nutrient intake (g/day) in sheep fed on diets contained deep-stacked broiler litter (DBL).

Items	Level of DBL in diet (g/kg DM)				SEM	P-value	
	0	70	140	210		Linear	Quadratic
DM	1150	1182	1345	1255	34.13	0.02	0.12
OM	1028	1076	1230	1119	34.32	0.04	0.07
CP	124	139	165	166	7.08	< 0.01	0.37
NDFom	622	635	724	651	15.93	0.053	0.03
ADFom	421	427	485	425	9.67	0.15	0.013
Copper (mg/day)	9.14	13.59	19.75	23.18	0.226	< 0.01	0.043
ME (MJ/day)	9.22	9.88	11.80	10.60	0.339	< 0.01	0.02

DM, dry matter; OM, organic matter; CP, crude protein; NDFom, ash-free neutral detergent fibre; ADFom, ash-free acid detergent fibre; SEM, standard error of means; ME, metabolisable energy.

Table 5
Effect of the experimental diets on apparent digestibility (g/kg) and estimated metabolisable energy (MJ/kg DM) in sheep fed on diets contained deep-stacked broiler litter (DBL).

Items	Level of DBL in diet (g/kg DM)				SEM	P-value	
	0	70	140	210		Linear	Quadratic
DM	544	567	610	603	13.2	0.006	0.32
OM	563	590	633	605	9.7	0.009	0.036
CP	595	660	674	666	16.6	0.030	0.10
NDFom	437	479	516	501	11.6	0.006	0.06
ADFom	429	461	500	470	17.0	0.11	0.15
ME	8.02	8.36	8.89	8.45	0.131	0.031	0.028

DM, dry matter; OM, organic matter; CP, crude protein; NDFom, ash-free neutral detergent fibre; ADFom, ash-free acid detergent fibre; ME, metabolisable energy; SEM, standard error of means.

the digestibility was not greater in the sheep fed the diet containing 210 g DBL/kg (diet 4) than the animals fed the 140 g DBL/kg DM diet. The minimum and maximum P-values of the quadratic effect of DBL rates on digestibility coefficients were P=0.036 for OM and P=0.32 for DM, respectively.

3.6. Purine derivatives and MPS

Allantoin, xanthine plus hypoxanthine, uric acid, total PD (TPD) excreted, TPD absorbed, and MPS increased (P<0.05) with the incorporation of increasing levels of DBL in diet (Table 6). Both linear and quadratic effects of DBL level on MPS were statistically significant. The MPS, which had shown a significant quadratic response, reached the greatest value in the sheep receiving DBL at the level of 140 g/kg diet DM (P<0.05). The statistical significance of the quadratic effect of DBL feeding on MPS was P=0.011.

Table 6
Effect of the experimental diets on urinary purine derivatives excretion (mmol/d) and microbial protein supply (MPS) (g/d) in sheep fed diets contained deep-stacked broiler litter (DBL).

Items	Level of DBL in diet (g/kg DM)				SEM	P-value	
	0	70	140	210		Linear	Quadratic
Purine derivatives							
Allantoin	4.55	6.55	6.90	7.00	0.375	<0.001	0.026
Uric acid	0.87	1.10	2.19	1.93	0.147	<0.001	0.12
X + H	0.38	0.54	0.68	0.62	0.030	<0.001	0.004
TPD excreted	5.81	8.17	9.80	9.55	0.445	<0.001	0.012
TPD absorbed	5.97	8.80	10.76	10.45	0.528	<0.001	0.011
MPS	27.14	39.99	48.90	47.50	2.403	<0.001	0.011

X + H, xanthine + hypoxanthine; TPD, total purine derivatives; SEM, standard error of means.

3.7. Ruminal parameters

Increasing amounts of DBL in the diet (Table 7) decreased the ruminal ammonia-N concentration and acetate to propionate ratio, and increased total VFA concentration (P<0.05). The counts of total protozoa and sub-family *Entoniniinae* decreased (P<0.05) as the level of DBL increased in the diet.

4. Discussion

4.1. Chemical composition

The fermentation process that occurred in the production of DBL resulted in degradation and losses of CP and NFC during deep stacking, which led to the proportional increases in the concentrations of ash and NDFom.

The increase in ash and the decrease in NFC content with increasing moisture content of DBL were due to the fact that the higher moisture content encourage degradation of OM in the fermenting mass (Raimbault, 1998).

4.2. Temperature of DBL

In this experiment, the elimination of pathogenic organisms at the depth of 30 cm with moistures 250 and 350 g/kg and the depth 60 cm with moisture 350 g/kg were observed because pathogens are eliminated when exposed to 55–60 °C for 30 min (Wassen and Strauch, 1976). This temperature was not recorded at depth 60 cm with moisture 250 g/kg and depth 120 cm. The increased temperature in DBL is due to the biological activity of microorganisms (*i.e.*, microbial growth and metabolism which generate heat) (Kwak et al., 2005). The greater temperature in the shallower depths of DBL (depths 30 and 60 cm) than the deeper depth (depth 120 cm) was due to higher levels of oxygen at the shallower depths stimulating the growth of aerobic microbes which generate heat (Kwak et al., 2005), and indicate more aerobic than anaerobic fermentation at the top

Table 7
Effects of the experimental diets on ruminal parameters and rumen protozoa (log_{10}/g digesta) in sheep fed diets contained deep-stacked broiler litter (DBL).

Items	Level of DBL in diet (g/kg DM)				SEM	P-value	
	0	70	140	210		Linear	Quadratic
Ruminal parameters							
pH	6.71	6.70	6.54	6.66	0.045	0.18	0.17
Ammonia-N (mg/dL)	18.6	16.6	13.5	14.6	1.13	0.012	0.20
Total VFA (mmol/L)	78.1	82.2	96.4	90.2	3.97	0.021	0.22
Acetate (mmol/L)	53.4	53.0	53.8	52.6	1.12	0.76	0.71
Propionate (mmol/L)	23.3	23.5	24.2	24.0	0.48	0.19	0.64
Butyrate (mmol/L)	16.6	17.1	16.8	17.5	0.91	0.59	0.91
Isovalerate (mmol/L)	3.34	3.18	2.64	2.88	0.231	0.08	0.40
Valerate (mmol/L)	3.38	3.16	2.55	2.99	0.196	0.06	0.11
Acetate:Propionate	2.87	2.57	2.07	2.26	0.206	0.027	0.25
Rumen protozoa							
Isotricha	2.83	2.66	2.66	2.73	0.325	0.68	0.41
Dasytricha	1.51	1.42	1.53	1.50	0.648	0.88	0.79
Entoniniinae	4.15	4.10	3.90	3.93	0.025	<0.001	0.12
Diplodiniiae	3.11	2.30	2.66	2.77	0.371	0.70	0.23
Ophryoscoleciniiae	1.50	1.49	1.51	1.53	0.620	0.96	0.97
Total protozoa	4.26	4.19	4.04	4.08	0.027	<0.001	0.08

SEM, standard error of means.
Ruminal parameters, values are averages of repeated sampling of rumen fluid collected from four animals assigned to each treatment just before lambs were offered the morning feeding (0 h) and 3 h after feeding.

(Bakshi and Fontenot, 1998). The results were similar to those reported by others (Chaudhry et al., 1998). Chaudhry et al. (1998) recorded maximum temperatures of 64 °C and 60 °C on day 7 for 80 and 40 cm depths, respectively. In another work, the temperature increased and reached its maximum (62 °C) on day 6 of deep stacking (Kwak et al., 2005). The differences in results reported in the studies could be related to some factors such as different deep stacking methods, capacity of stack and mean ambient temperature.

Despite our study, in the experiment conducted by Chaudhry et al. (1998), the temperatures of DBL (*i.e.*, 42 and 46 °C for 80 and 40 cm depths, respectively) were only slightly higher than the ambient temperature.

Bucklin et al. (2012) recommended that to achieve the appropriate heating to eliminate pathogens, the moisture content of BL should be 200–250 g/kg and the stacking depth from 183 to 244 cm. In our study, however, the temperatures of DBL containing both 250 and 350 g moisture/kg did not reach the appropriate level for eliminating pathogens at the depth of 120 cm. The temperature of DBL with 350 g moisture/kg reached its maximum sooner than that of DBL with 250 g moisture/kg. After reaching the maximum temperature level, the gradual decline of temperature in DBL was similar at the three depths, and was similar to that reported by Bakshi and Fontenot (1998), but there was a different pattern of temperature decline between moisture levels of 250 and 350 g/kg.

4.3. Biological analysis

The standard for determining the effectiveness of processing methods on the safety of BL are that it should result in less than 20,000 bacteria and less than 10 *Coliforms* per gram of sample by plate count (Caswell et al., 1975). The values observed in our study were in the safety ranges outlined above. This was due to heat produced during fermentation of DBL which was sufficient to kill any pathogenic bacteria (Kwak et al., 2005). The elimination of *Coliform* bacteria in DBL containing 350 g moisture/kg at depths 30 and 60 cm could, in part, be due to sensitivity of these bacteria to temperatures above 42 °C (McCaskey et al., 1985) and also the accumulation of ammonia-N released from the breakdown of uric acid during the deep-stacking process (McCaskey and Martin, 1988). Elimination of *Coliforms* with the deep stacking of BL was in line with the findings of other researchers (Chaudhry et al., 1998).

Factors such as temperature, ammonia concentration, pH, moisture and competition from indigenous microorganisms were potential factors affecting *Salmonella* survival (Bush et al., 2007). The decline occurred in the *Salmonella* population in our study was in agreement to that result reported by Kwak et al. (2005). They, also, reported that *Salmonella* and *E. coli* were eliminated in a shorter period in DBL compared to non-stacked BL.

In the present study, the pathogenic bacteria populations observed in the stack containing 250 g moisture/kg and at depth 120 cm could be due to it reaching a lower temperature compared to the others. In other word, deep stacking with low moisture (250 g moisture/kg) had not decreased the pathogens in DBL to a safe level to feed to ruminants. The higher concentration of *E. coli* for depth of 120 cm and moisture of 250 g/kg compared to the other treatments was related to the lower maximum temperature (Fid. 1) in the former (Ruffin and McCaskey, 1990). In farm conditions, therefore, a practical approach to obtain an appropriate DBL could be the deep stacking BL with 350 g moisture/kg. Bakshi and Fontenot (1998) concluded that deep stacking BL with 300 or 400 g moisture/kg eliminated pathogenic organisms. As reported by Ruffin and McCaskey (1990), temperatures above 54 °C effectively eliminate pathogenic bacteria including *Coliforms*, *E. coli* and *Salmonella*.

4.4. Nutrients intake

The increased feed intake in the sheep receiving DBL up to 140 g/kg diet DM compared to those fed the control diet was due to the increasing digestibility coefficients of diet (Table 5). However, the tendency towards a quadratic effect of different DBL levels on intake in our study indicates feeding DBL at more than 140 g/kg dietary DM may have an adverse effect on diet palatability. Rossi et al. (1998) reported an increase in feed intake when DBL was offered to Holstein steers. In contrast, Negesse et al. (2007) reported that feeding DBL in the diet of Spanish wethers decreased the intakes of DM, OM and CP. The discrepancy between these studies may be due to different experimental animals, diets and the composition of DBL used, all of which may affect experimental responses (McCaskey et al., 1989; Givens et al., 2000).

4.5. In vivo apparent digestibility of nutrients

Improving *in vivo* digestibility coefficients of the nutrients when adding up to 140 g of DBL per kg diet compared to the control diet could be associated with the greater DM digestibility (DMD) of BL (781 g/kg; Badiee-Baghsiah et al., 2013) when substituted for alfalfa (DMD, 650 g/kg) and wheat straw (DMD, 281 g/kg) in the diet. Another reason for the enhancement of digestibility with increasing dietary level of BL could be due to the rising CP levels and declining NDFom content of the diets and their effect on digestibility (Van Soest, 1994). Higher NDF digestion in sheep fed DBL diets compared to those fed on the control indicates that BL may have increased the rate of digestion of hay fibre (Mekasha et al., 2004). Belanche et al. (2012b) indicated that diets with low CP concentration have a negative impact on the numbers of fibrolytic microbes in the rumen.

The decrease in the digestibility coefficients with the addition of 210 g of DBL/kg dietary DM compared to 140 g DBL/kg diet DM could be due to the palatability and intake of this by-product. Indeed, in this study the major part of the observed

ort in the animals fed 210 g DBL/kg diet DM consisted of DBL, which resulted in the enhancement of alfalfa and wheat straw ratio in the consumed ration and thus a decline in digestibility. However, the diet digestibility in the sheep fed 210 g DBL/kg DM was greater than that in the control group.

In the experiment conducted by Mekasha et al. (2004), *ad libitum* feeding of DBL to wethers increased digestibility. In contrast to our results, Bakshi and Fontenot (1998) and Negesse et al. (2007) reported declines in the digestibility of DBL diets compared to a control diet. However, Negesse et al. (2007) concluded that feeding Spanish wethers with DBL increased NDF digestibility, but CP digestibility was reduced. The different results may be due to differences in ingredients and composition of experimental diets, composition of DBL and the level of BL used in different studies (McCaskey et al., 1989).

4.6. Purine derivatives and MPS

The improved MPS in the animals fed diets containing up to 140 g/kg of DBL compared to those fed the control diet could be due to increased intakes of DM and CP (Rezaei et al., 2014). Higher DM digestibility, and thereby increased daily ME intake, and greater CP supplied by diets, may have resulted in better synchrony of ME and N in the rumen of sheep fed the diets containing up to 140 g DBL/kg DM. The greater MPS of the diets containing up to 140 g DBL may have resulted from increased CP digestibility (Ørskov, 1982).

The decline of MPS when feeding the animals the diet containing 210 g DBL/kg DM compared to 140 g DBL/kg DM might be associated with decrease in diet digestibility and ME intake that resulted lower ruminal synchronization of nutrients to microbial production.

4.7. Ruminal parameters

The ruminal pH values were in a normal range (6.1–6.8) described by Van Soest (1994). According to Elemam et al. (2009), ruminal pH in sheep fed DBL was lower than those fed on a control diet. Feeding DBL in sheep and buffalo steers increased the pH of rumen fluid (Chaudhry et al., 1996; Chaudhry and Naseer, 2012). The results among these studies were different due to various diet ingredients and the level of DBL used.

The concentrations of ruminal ammonia-N in all the treatments were between 8.5 and 30 mg/dL, which according to McDonald et al. (2011) is optimum range for microbial growth.

The change that occurred in the concentration of ammonia-N in the rumen with added DBL in diet was associated with the change in MPS and uptake of ammonia-N by microbes for protein synthesis (Azizi-Shotorkhoft et al., 2012). Another reason for the reduction of rumen ammonia with added DBL in the diet could be related to the decline of total protozoa numbers in the rumen because a typical protozoal population is able to break down about 17% of available rumen bacteria every hour (Belanche et al., 2012a). Elemam et al. (2009) reported a higher ruminal concentration of ammonia in lambs fed on increasing dietary levels of DBL. The decline of ruminal ammonia-N concentration in this study was similar to results reported by Hopkins and Poore (2001) when replacing soybean meal and cottonseed meal with DBL. According to Hopkins and Poore (2001), ruminal ammonia is generally positively associated to CP degradability. Degradability of DBL should be high due to its greater non protein nitrogen content (>400 g/kg CP), though excessive heat produced during the deep stacking process (>50 °C for long time) may increase the level of unavailable CP (acid detergent insoluble nitrogen), which decreases CP degradability to some extent.

The greater intakes of DM and OM and greater OM digestibility caused more fermented OM per day in the rumen of sheep fed the diets containing DBL compared to those fed the control diet. This could be responsible for the increased concentration of ruminal total VFA in the former (McDonald et al., 2011). The result was similar to that reported by Chaudhry and Naseer (2012) in buffalo steers. In the work by Chaudhry et al. (1996), total VFA concentration in the rumen of sheep was increased insignificantly by inclusion of DBL in diet. In the present study, the maximum ruminal total VFA was observed in the sheep fed 140 g DBL/kg diet DM, and the VFA concentration changes were in parallel to the changes of feed intake and diet digestibility.

Similar to our results, the decline of ruminal acetate to propionate ratio with increasing DBL in diet was reported by Rossi et al. (1998). In contrast, Chaudhry et al. (1996) reported an increase in acetate to propionate ratio in the rumen of sheep fed on a diet that included DBL compared to those fed on a control diet.

The reduction of protozoa in the rumen of sheep fed the diets containing DBL compared to the control group might be associated with decreasing hay levels in the diet with the addition of DBL, because dried grass or hay may increase the growth of rumen protozoa (Eadie et al., 1967). Moreover, the higher Cu content in the diets containing DBL compare to free DBL diet may lead to decrease the ruminal protozoa population in sheep as noted by Kišidayová et al. (2000) and Vardyova et al. (2006). They reported that Cu has a toxic effect on ruminal protozoa population in sheep.

5. Conclusion

To obtain a safe feedstuff, BL should be deep stacked at 350 g moisture/kg, which leads to a decrease in pathogenic bacteria numbers to the safe level for ruminant consumption. Although, the maximum animal response was observed in the sheep fed 140 g DBL/kg diet DM, feeding the animals with DBL containing 350 g moisture/kg, up to 210 g/kg diet DM, improved feed

intake, digestibility, MPS and ruminal fermentation compared to the control group, without any adverse effects on animal health. Moreover, the use of BL as a feedstuff can reduce feeding costs and environmental pollution.

Conflict of interest

The authors wish to confirm that there are no known conflicts of interest associated with this publication and there has been no financial support for this work that could have influenced its outcome.

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References

Agricultural Food Research Council (AFRC), 1993. Energy and Protein Requirements of Ruminants. Technical Committee on Responses to Nutrients. CAB International Publication, Wallingford, UK.
AOAC, 1990. Association of Official Analytical Chemists, Official Methods of Analysis, 15th ed. AOAC, Arlington, VA, USA.
APHA., 1998. Standards Methods for the Examination of Water and Wastewater, 20th ed. American Public Health Association, Washington, DC.
Azizi-Shotorkhoft, A., Rouzbehan, Y., Fazaeli, H., 2012. The influence of the different carbohydrate sources on utilization efficiency of processed broiler litter in sheep. Livest. Sci. 148, 249–254.
Badiee-Baghsiah, M., Rouzbehan, Y., Fazaeli, H., Rezaei, J., 2013. Effect of heat processing on chemical composition, protein fractions and digestibility of broiler litter. Iran. J. Anim. Sci. 1, 9–21.
Bakshi, M.P.S., Fontenot, J.P., 1998. Processing and nutritive evaluation of broiler litter as livestock feed. Anim. Feed Sci. Technol. 74, 337–345.
Belanche, A., de la Fuente, G., Moorby, J.M., Newbold, C.J., 2012a. Bacterial protein degradation by different rumen protozoal groups. J. Anim. Sci. 90, 4495–4504.
Belanche, A., Doreau, M., Edwards, J.E., Moorby, J.M., Pinloche, E., Newbold, C.J., 2012b. Shifts in the rumen microbiota due to the type of carbohydrate and level of protein ingested by dairy cattle are associated with changes in rumen fermentation. J. Nutr. 142, 1684–1692.
Broderick, G., Kang, J.H., 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and *in vitro* media. J. Dairy Sci. 54, 1176–1183.
Bucklin, R.A., Jacob, J.P., Nordstedt, R.A., Sloan, D.R., Tervola, R.S., Mather, F.B., 2012. Storage of Broiler Litter. Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, Univ. Florida. PS15, pp. 3.
Bush, J., Poore, H., Rogers, M., Altier, C., 2007. Effect of stacking method on *Salmonella* elimination from recycled poultry bedding. Bioresour. Technol. 98, 571–578.
Caswell, L.F., Fontenot, J.P., Webb Jr., K.E., 1975. Effect of processing method on pasteurization and nitrogen components of broiler litter and on nitrogen utilization by sheep. J. Anim. Sci. 40, 750–759.
Chaudhry, S.M., Fontenot, J.P., Naseer, Z., Ali, C.S., 1996. Nutritive value of deep stack broiler litter for sheep. Anim. Feed Sci. Tech. 57, 165–173.
Chaudhry, S.M., Fontenot, J.P., Naseer, Z., 1998. Effect of deep stacking and ensiling broiler litter on a chemical composition and pathogenic organisms. Anim. Feed Sci. Technol. 74, 155–167.
Chaudhry, S.M., Naseer, Z., 2012. Processing and nutritional value of broiler litter as a feed for buffalo steers. J. Anim. Plant Sci. 22, 358–364.
Chen, X.B., Gomes, J.M., 1992. Estimation of Microbial Protein Supply to Sheep and Cattle Based on Urinary Excretion of Purine Derivatives: An Overview of the Technical Details. International Feed Resources Unit Rowett Res. Inst. (Occasional publication), Bucksburn, Aberdeen, UK, pp. 21.
Collee, J.G., Fraser, A.G., Marmion, B.P., Simmons, A., 1996. Mackie and McCartney Practical Medical Microbiology, 14th ed. Churchill Livingstone, New York.
Deshck, A., Abo-Shehada, M., Allonby, E., Givens, D.I., Hill, R., 1998. Assessment of the nutritive value for ruminants of poultry litter. Anim. Feed Sci. Technol. 73, 29–35.
Eadie, J.M., Hobson, P.N., Mann, S.O., 1967. A note on some comparisons between the rumen content of barley-fed steers and that of young calves also fed on a high concentrate ration. Anim. Prod. 9, 247–250.
Elemam, M.B., Fadelelseed, A.M., Salih, A.M., 2009. Growth performance, digestibility, N-balance and rumen fermentation of lambs fed different levels of deep-stack broiler litter. Res. J. Anim. Vet. Sci. 4, 9–16.
Givens, D.I., Owen, E., Axford, R.F.E., Omed, H.M., 2000. Forage Evaluation in Ruminant Nutrition, 1st ed. CABI Publ., Wallingford, UK, pp. 480.
Holt, J.G., 1994. Bergey's Manual of Determinative Bacteriology, 9th ed. Williams & Wilkins, Baltimore, USA.
Hopkins, B.A., Poore, M.H., 2001. Deep-stacked broiler litter as a protein supplement for dairy replacement heifers. J. Dairy Sci. 84, 299–305.
Kišidayová, S., Sviatko, P., Zeleňák, I., 2000. The effect of copper and cobalt supplement at ion on the rumen ciliate population in sheep. Czech J. Anim. Sci. 45, 345–348.
Kwak, W.S., Huh, J.W., McCaskey, T.A., 2005. Effect of processing time on enteric bacteria survival and on temperature and chemical composition of broiler litter processed by two methods. Bioresour. Technol. 96, 1529–1536.
Mavimbela, D.T., Van Ryssen, J.B.J., 2001. Effect of dietary molasses on the site and extent of nutrients in sheep fed broiler litter. South African J. Anim. Sci. 31 (1), 33–39.
McCaskey, T.A., Anthony, W.B., 1979. Human and animal health aspects of feeding livestock excreta. J. Anim. Sci. 48, 163–177.
McCaskey, T.A., Martin, J.B., 1988. Evaluation of a process for improved quality and microbial safety broiler litter. Biol. Wastes. 25, 209–218.
McCaskey, T.A., Stephenson, A.H., Ruffin, B.G., 1989. Good management necessary to cash in on broiler litter resource. Agric. Res. 36 (3), 14.
McCaskey, T.A., Sutton, A.L., Lincoln, E.P., Dobson, D.C., Fontenot, J.P., 1985. Safety aspects of feeding animal waste, utilization and management. In: Proc. 5th Int. Symposium on Livestock Wastes, 16-17 December. American Society of Agricultural and Biological Engineers Publication, Chicago, IL, pp. 275–285.
McDonald, P., Edwards, R.A., Greenhalgh, J.F.D., Morgan, C.A., Sinclair, L.A., Wilkinson, R.G., 2011. Animal Nutrition, 6th ed. Prentice Hall, Essex, UK.
Mekasha, Y., Merkel, R.C., Goetsch, A.L., Sahlu, T., Tesfai, K., 2004. Effects of method of offering broiler litter and level of prairie hay intake on growth of Boer × Spanish wethers. Small Rumin. Res. 55, 123–133.
Negesse, T., Patra, A.K., Dawson, L.J., Tolera, A., Merkel, R.C., Sahlu, T., Goetsch, A.L., 2007. Performance of Spanish and Boer × Spanish doelings consuming diets with different levels of broiler litter. Small Rumin. Res. 69, 187–197.
NRC, 2007. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervidae, and New World Camelids, 1st ed. Natl. Res. Council, Natl. Academic Press, Ashington, DC, USA, pp. 362.
Ørskov, E.R., 1982. Protein Nutrition in Ruminants, 2nd ed. Academic press Inc., London.
Rezaei, J., Rouzbehana, Y., Fazaeli, H., Zahedifar, M., 2014. Effects of substituting amaranth silage for corn silage on intake, growth performance, diet digestibility, microbial protein, nitrogen retention and ruminal fermentation in fattening lambs. Anim. Feed Sci. Technol. 199, 29–38.
Robertson, J.B., Van Soest, P.J., 1981. The detergent system of analysis and its application to human foods. In: James, W.P.T., Theander, O. (Eds.), The Analysis of Dietary Fiber in Food. Marcel Dekker, NY, USA, pp. 123–158 (Chapter 9).

Rossi, J.E., Goetsch, A.L., Galloway, D.L., 1998. Intake and digestion by Holstein steers consuming different particle size fractions of broiler litter. Anim. Feed Sci. Technol. 71, 145–156.
Ruffin, B.G., McCaskey, T.A., 1990. Broiler litter can serve as feed ingredient for beef cattle. Feedstuffs 62, 13–17.
Raimbault, M., 1998. General and microbiological aspects of solid substrate fermentation. E. J. Biotechnol. 1, 174–188.
Statistical Centre of Iran, 2013. Selected Census Results of the Broiler Chicken Farms, the Year 1391 (March/21/2012 to March/20/). Vice–Presidency for Strategic Planning & Supervision, Statistical Centre of Iran, pp. 34.
Van Soest, P.J., 1994. Nutritional Ecology of the Ruminant. Comstock Publication, Ithaca, NY, USA.
Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber and non starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.
Vardyova, Z., Mihalikova, K., Kisidayova, S., Javorsky, P., 2006. Fermentation pattern of the rumen and hindgut inocula of sheep grazing in an area polluted from the non-ferrous metal industry. Czech J. Anim. Sci. 51, 66–72.
Wassen, H., Strauch, D., 1976. Treatment of liquid animal and communal by-products by rotation method (System Fuchs). Berl. Muench. Tieraerztl. Wochenschr. 89, 96–100 (in German).