Effect of meal mixes properties on extrusion process and pellet quality of aquafeed formulated with three different fish meals or feather meal levels

Rolland M, Hjermitslev NH, Orlien V, Olsen K, Rasmussen MA, Rasmussen HT

Rolland M, Hjermitslev NH, Orlien V, et al. Effect of meal mixes properties on extrusion process and pellet quality of aquafeed formulated with three different fish meals or feather meal levels. J Aquac Fisheries Manage. 2018;1(1):5-15.

ABSTRACT

The present study aimed at modelling the effect of meal mix properties, measured by the water soluble protein (WSP) and the water holding capacity (WHC), on the extrusion system response and the pellet quality of aquafeeds. For this purpose, six diets were formulated using three quality fish meal and three inclusion levels of hydrolysed feather meal (8, 16 and 24%). The meal mixes were extruded at different levels of temperature (around 100, 110 or 130°C) and moisture (around 22, 25 or 28%), using a twin screw extruder. The response of the extruder was characterized by the specific mechanical energy (SME), specific thermal energy (STE) and screw back pressure (SBP). The pellet quality was defined as the radial expansion index, bulk density, retention, oil absorption capacity, oil leakage and starch gelatinization. The data were analysed using the flexible framework of generalized additive models (GAMs). The results showed that the SME increased with increasing WSP ($P<0.001$) while STE decreased ($P<0.001$). The SBP was only affected by the process parameters; increased T led to increased SBP while moisture had the opposite effect ($P<0.01$). The pellet quality variables were affected by both the meal mix properties and the process parameters, often in interaction. In general the models explained between 64.1 and 95.4% of the variance, and showed that WSP and WHC described well the meal mix effect on the variables investigated. Replacing WSP and WHC by the diet as a factor in the GAMs led to significant decrease in the variance explained for bulk density (88.4 and 31.3%) and oil absorption capacity (65.1 and 44.4%) because of the inability to model the interactions. The results suggest that, WSP and WHC could explain to some extent the effect of meal mixes on extrusion system response and pellet quality indicators.

Key Words: *Protein properties; Water soluble fraction; Water holding capacity; Extrusion process; General additive model*

INTRODUCTION

Nutrient-dense aquafeeds are typically produced by extrusion process. This allow for creating cylindrical-shaped pellets with a porous structure that are able to retain an oil coat. In addition to meeting the nutritional requirement of the fish, the pellet must display adequate physical properties in order to fulfil the commercial requirements. Properties such as sinking velocity, durability through handling and transport, water stability and oil retention will have a strong effect on the feed performance and commercial value (1).

Because of the development of large fish farms, the handling, transport and storage practices has changed towards bulk delivery, more efficient economically and environmentally than bags. However, such a shift leads to increased physical stress on the pellets (2). In addition, handling in large fish farms is typically achieved through pneumatic conveying and rotor spreading systems. During feeding, pellets are subjected to attrition causing breakage or chipping. This can represent a direct economic loss for the farmers as small particles will not be eaten by the fish and risk to clogs the conveying tubes (3,4). Besides, small particles will contribute to water pollution. Altogether, the physical properties of pellets are of major importance regarding the quality of aquafeeds. In addition, the diversity of the raw materials and the nutritive constraints also influence quality and further require needs for controlling the extrusion (1).

Starch is the factor responsible for binding pellet components together but also for the expansion property and the hardness of the pellet (5,6). However, protein and lipids are more nutritionally efficient than carbohydrates for maximizing growth, and it is of interest to reduce the starch content in diets for carnivorous species. Such replacement calls for more knowledge about the functional properties of the other nutrients (1,7,8). Feed formulation is based on different types of proteins originating from fish, plant or animal meal, which differs in their nutritional values. Proteins constitute up to 50% of a carnivorous fish diet and high substitution of fish meal (FM) with plant or land-based animal proteins, allow for the production of more sustainable aquafeeds without affecting growth performances of carnivorous species (9-13).

Different proteins have different techno-functional properties and changes in the proteinaceous fraction have major influence on the process parameters and the resulting pellet quality (14,15). Fish meal has distinct properties compared to plant proteins in aquafeeds production (14,16). The literature on processing of feed based on proteins originating from land-based animal is scarce, thus the increasing interest in such protein warrant more research. The prices for protein material such as feather meal are very low due to large available volume and little interest from other sectors such as human food production. Feather meal (FeM) consists of approximatively 80% proteins, mainly keratins. Keratins have a primary chain of 10.2-10.4 kDa associated in strong fibrous proteins by hydrophobic interactions and disulphide bounds (17).

The structure of keratins makes it difficult to digest and valorise (18,19). Pre-processing, such as hydrolysis or steam explosion, will alter the protein structure and is likely to improve its nutritional value for fish (17,18,20).

The present study investigated whether properties of three different FM types (specie and pre-processing) and hydrolysed FeM could account for the changes on extrusion system response and pellet quality. Water soluble protein (WSP) and water holding capacity (WHC) are important functional properties affecting both the process and the pellet quality (15,21). The WSP is composed of peptides and amino acids that are expected to act as a plasticizer (16,21). Plasticizers are defined as “compound that imparts a desirable degree of flexibility over a broad range of use temperatures and lowers the brittle point” (22). The WHC was expected to relate to the amount of water that, after protein hydration, would remain to plasticize other ingredients (15).

Department of Chemisstry, Univercity of Copenhagen, Frederiksberg, Denmark.

Correspondence: Marine Rolland, Department of Chemisstry, Univercity of Copenhagen, Frederiksberg, Denmark, Telephone: 335 45 90 35, e-mail marol@biomar.fr

Received: May 30, 2018; Accepted: July 03, 2018; Published: July 10, 2018

The WSP and WHC were therefore hypothesized to be major raw material properties susceptible to affect the extrusion system response and pellet quality. For this purpose, six different meal mixes were extruded at different temperature and moisture levels and the data collected on the extrusion system response and pellet quality were analysed by general additive models (GAMs). In addition, to investigate the effect of other raw material properties not accounted for by WSP and WHC, the data analysis was also conducted using Diet as a factor.

MATERIALS AND METHODS

Feed ingredients and production

The protein, fat and moisture content for the three FMs, the FeM and the wheat flour (WF) are given in Table 1. Six recipes based on three different types of FM and three levels of FeM were designed (Table 2). The FMs were originating from North Altantic fisheries, mainly herring. FM1 and FM2 were produced after intense drying treatment using steam panels. FM3 was produced under more gentle conditions, i.e. vacuum dried at maximum 70°C. The FeM was a commercial available feather meal hydrolysate (GePro, Germany). Wheat flour was used as the source of starch (Table 2). The diets were formulated to fulfil the nutritive requirements of rainbow trout (9).

The feed were produced at BioMar A/S Tech Center, Brande, Denmark over a three day trial. Dry ingredients were milled in a hammer mill with a screen size of 0.75 mm and mixed. The meal mixes were extruded with a twin screw-extruder BC 45 (Clextral, France). Three different temperature levels, around 100, 110 and 130 °C and three moisture contents (around 22, 25 and 28%) were applied to the different diets resulting in the production of 26 extrudates (Table 3). The temperature was measured just before the die using an inbuilt probe. The steam addition was kept constant at 10 kg/h so the changes in moisture level were only due to water.

The screw configuration was set up with a reverse section after the feeding zone followed by a transport zone passing the degasser and lastly a pressure built up towards the die plate. The mass flow was set at a constant rate of 140 kg/h. For the present trial the die diameter was 2.4 mm and, there were 4 or 8 die holes on the plate. To monitor the extruder response to the experimental conditions, the specific mechanical energy (SME, kJ/kg), the specific thermal energy (STE, kJ/kg) and screw back pressure (SBP, bar) were measured for each production batch when the system was stabilized to desired temperature and moisture levels. The specific mechanical energy is the energy transmitted to the dough by shear forces of the screw, while the specific thermal energy is the energy brought to the dough by the steam, the water or the barrel heating. Finally the screw back pressure is the pressure applied by the dough on the screw at the die plate. The meal mixes were sampled before entering the extruder. The extrudates were sampled at the die and the final pellets were sampled after going through drying, oil coating and sifting.

Meal mixes and extrudates properties

The six different meal mixes and the extrudates were analysed for physicochemical properties. The meal mixes were directly analysed, while the

TABLE 1
Proximate composition, water soluble protein (WSP) and water holding capacity (WHC) of the raw materials

	Fish Meal NA Poor quality	Fish Meal NA Standard quality	Fish Meal NA LT Good quality	Feather meal	Wheat Flour
Dry matter (g/kg)	95,40	93,50	91,90	95,10	86,90
Protein (g/kg DM)	73,17	75,19	73,99	88,64	12,77
Fat (g/kg DM)	13,73	12,19	14,25	10,41	2,42
WSP (% total protein)	8,94	7,06	7,14	3,70	15,73
WHC (g /g DM)	1,15	1,41	1,36	1,36	0,90

TABLE 2
Recipes and Nutrient composition for the six experimental diets, before extrusion, after extrusion and after oil coating

	FM 1	FM 2	FM 3	FeM 24	FeM 16	FeM 8
Recipe (%)						
Fish Meal NA Bad	53.13					
Fish Meal NA STD		53.10				
Fish Meal NA LT Skagen			52.57	20.67	31.54	42.84
Feather Meal				24.00	16.00	8.00
Wheat Flour	18.50	18.00	19.70	24.07	23.50	21.00
L-Lysine HCl				1.08	0.48	
DL-Methionine				0.37	0.19	
L-Histidine	0.08	0.08	0.16	0.66	0.48	0.31
L-Tryptophan					0.05	0.01
Mono-calcium Phosphate				0.7		
Yttrium	0.05	0.05	0.05	0.05	0.05	0.05
Fish Oil	8.30	8.50	6.40	6.50	6.40	6.40
Rapeseed Oil	17.20	17.70	19.10	19.50	19.20	19.30
Nutritient composition of the meal mixes[a](g/kg)						
Protein	530	532	512	526	533	536
Lipid	92	79	91	75	77	86
Moisture	75	86	95	86	86	92
Nutritient composition of the extrudates[b] (g/kg)						
Protein	555 ± 5	560 ± 10	533 ± 10	547 ± 14	5470 ± 11	530 ± 3
Lipid	96 ± 1	84 ± 2	95 ± 2	76 ± 1	81 ± 2	89 ± 1
Moisture	54 ± 4	68 ± 2	69 ± 29	70 ± 23	67 ± 22	94 ± 11
Nutritient composition of the coated pellets[c] (g/kg)						
Protein	404 ± 2	412 ± 5	399 ± 5	402 ± 7	40.30 ± 0.70	397 ± 15
Lipid	343 ± 1	338 ± 4	347 ± 2	325 ± 14	33.60 ± 0.30	337 ± 1
Moisture	55 ± 7	44 ± 8	48 ± 6	50 ± 2	3.90 ± 1.10	71 ± 12

FM: Fish Meal; FeM: Feather Meal; asampled after meal mixer and before pre-conditioning; bsampled at the end of the extruder; csampled after sifter. The values for extrudates and coated pellets are given as the mean ± SD for a same recipe undergoing the different moisture and temperature during process.

TABLE 3
Results for the meal mix and extrudates physico-chemical properties, extrusion parameters, system response and pellet quality indicators for the 26 production batches

Batch number	Diet	Physico-chemical properties		Extrusion parameters		System reponse			Pellet quality indicators					
		WSP (%)	WHC (g/g DM)	Temperature (°C)	Moisture (%)	SME (kJ/kg)	STE (kJ/kg)	SBP (bar)	Expansion Index (%)	Bulk Density (g/L)	Retention (%)	Starch Gelatinization (%)	Oil Absorption Capacity (%)	Oil Leaking (%)
Meal mix	FM1	10.92 ± 0.19	0.91 ± 0.00											
1	FM1	9.39 ± 0.59	1.72 ± 0.09	94.00	25.20	48.10	64.00	12.00	142.10	429.80	69.00	26.00	24.67	0.25
2	FM1	9.42 ± 0.41	1.56 ± 0.01	90.50	28.00	36.00	72.90	8.00	147.00	340.20	nd	22.10	nd	nd
3	FM1	9.60 ± 0.04	1.56 ± 0.01	119.80	25.20	48.70	62.10	20.00	136.60	479.60	42.00	47.1	32.70	0.20
4	FM1	9.94 ± 0.22	1.47 ± 0.02	125.40	25.20	44.00	65.90	15.00	152.10	459.50	66.00	40.30	34.90	0.00
Meal mix	FM2	8.72 ± 0.02	10.8 ± 0.00											
5	FM2	7.38 ± 0.18	1.70 ± 0.06	100.70	27.80	44.00	74.60	11.00	166.70	452.70	56.00	24.70	32.90	0.61
6	FM2	7.48 ± 0.23	1.78 ± 0.11	110.0	25.20	39.70	nd	17.00	141.90	459.30	65.00	26.20	34.70	0.19
7	FM2	7.32 ± 0.11	2.23 ± 0.05	131.30	25.20	38.40	63.90	14.00	146.30	472.90	70.00	27.20	33.4	0.40
Meal mix	FM3	9.18 ± 0.20	1.01 ± 0.00											
8	FM3	8.14 ± 0.40	1.77 ± 0.08	95.10	24.90	42.40	64.00	18.00	156.70	442.80	65.00	21.10	35.00	0.00
9	FM3	7.33 ± 0.35	1.75 ± 0.04	95.00	28.00	nd*	nd	nd	149.30	467.20	nd	15.70	nd	nd
10	FM3	7.72 ± 0.08	1.80 ± 0.08	110.00	24.30	45.90	62.20	18.00	151.60	439.50	73.00	19.30	34.30	0.20
11	FM3	7.49 ± 0.07	1.69 ± 0.06	128.40	24.90	40.70	61.60	13.00	151.00	437.50	72.00	25.20	34.70	0.00
Meal mix	FeM24	8.20±0.06	0.90±0.01											
12	FeM24	6.02 ± 0.14	1.58 ± 0.02	104.30	24.90	43.20	91.60	15.00	129.20	477.00	nd	44.00	nd	nd
13	FeM24	6.56±0.06	1.60 ± 0.03	102.80	28.00	42.00	89.50	13.00	133.10	466.10	3.00	25.10	32.30	3.27
14	FeM24	6.55 ± 0.11	2.12 ± 0.10	108.00	25.50	40.60	86.40	8.00	127.10	436.90	28.00	27.90	35.30	1.72
15	FeM24	7.00 ± 0.21	1.59 ± 0.01	128.70	25.70	38.70	67.80	11.00	117.70	459.40	23.00	37.60	35.00	1.74
Meal mix	FeM16	8.23 ± 0.03	0.94 ± 0.00											
16	FeM16	6.58 ± 0.02	1.66 ± 0.05	103.90	25.10	42.90	91.40	21.00	127.10	483.40	nd	30.60	nd	nd
17	FeM16	6.15 ± 0.36	1.66 ± 0.05	98.50	27.60	41.90	88.10	12.00	136.50	459.10	5.00	26.10	34.70	1.55
18	FeM16	6.34 ± 0.07	2.12 ± 0.07	113.20	25.40	34.40	105.20	11.00	137.30	453.60	62.00	25.30	32.30	1.64
19	FeM16	6.98 ± 0.11	1.61 ± 0.05	130.00	21.80	40.40	104.80	23.00	123.60	473.00	nd	50.80	nd	nd
20	FeM16	6.88 ± 0.07	1.79 ± 0.02	129.50	25.10	33.60	102.30	12.00	140.70	473.40	62.00	25.00	30.00	0.22
Meal mix	FeM8	8.40 ± 0.06	0.96 ± 0.01											
21	FeM8	6.96 ± 0.20	2.22 ± 0.02	95.00	25.00	nd	nd	nd	121.30	nd	nd	25.80	nd	nd
22	FeM8	6.94 ± 0.21	2.09 ± 0.11	95.80	28.90	43.80	119.00	12.00	121.40	nd	34.00	21.90	30.70	2.56
23	FeM8	6.79 ± 0.01	1.90 ± 0.03	122.20	24.60	35.40	107.40	18.00	152.50	452.80	74.00	29.60	33.30	0.80
24	FeM8	7.05 ± 0.18	1.58 ± 0.03	142.20	22.10	52.90	114.20	43.00	133.30	467.50	nd	44.10	nd	nd
25	FeM8	7.26 ± 0.14	1.71 ± 0.03	137.10	25.50	43.40	108.20	26.00	134.70	493.30	nd	37.80	nd	nd
26	FeM8	7.21 ± 0.03	1.71 ± 0.03	125.20	27.70	36.60	105.70	12.00	144.20	454.50	68.00	30.00	30.30	0.65

*Not determined; WSP: water soluble protein; WHC: water holding capacity; SME: specific mechanical energy; STE: specific thermal energy; SBP: screw back pressure.

extrudates were grinded using a coffee grinder (OBH Nordica, Denmark) for 10s in order to obtain a homogenous powder. The dry matter (DM) content of each sample was measured by the weight difference between the sample before and after oven drying (105°C for 16 h).

The water holding capacity (WHC) was measured by dispersion 5 g of sample into 25 ml of distilled water in pre-weighted centrifuged tubes and shaken for 10 min, following centrifugation for 30 min at 1664 g. The supernatant was discharged and the tube was weighted. The WHC (g H_2O /g DM) was calculated as follow:

$$\mathrm{WHC} = \frac{\mathrm{Wfinal - Wtube - Wsample}}{\mathrm{Wsample}}$$

Where Wfinal is the weight (g) of the tube after the supernatant is discharged, Wtube is the weight (g) of the empty tube and Wsample is the amount (g) of sample in the tube. For both DM and WHC each sample was analysed in duplicates.

The water soluble protein fraction (WSP) was measured by dispersing 0.5 g of sample in 10 ml of distilled water using centrifuge tubes and shaken for 2 h. The tubes were then centrifuged at 2105 g for 10 min and the protein content of the supernatant was measured using a BCA protein assay (Pierce Biotechnology Inc, Rockford, USA) according to manufacturer recommendation and bovine serum albumin as standard. The total protein content (Nx 6.25) in the sample was determined by combustion using a FP628 Nitrogen/Protein (LECO Corporation, St. Joseph, US). The WSP (% of total protein content) was calculated as follow:

$$\mathrm{WSP} = \frac{\text{Protein content in supernatant}}{\text{Total protein content in the sample}} \times 100$$

Each sample was analysed in duplicates. It should be mentioned that two different methods were used to measure the total protein content or the soluble protein fraction (Kjeldahl or BCA protein assay). This was not expected to have a major effect on the results as both methods have been showed to correlate (23).

Pellet quality indicators

The expansion index (EI) and bulk density (BD) of the extrudates were measured on randomly collected samples at the extruder die (diameter of 2.4 mm) after the process parameters were stabilized to desired temperature and moisture levels. The EI (%) was measured on 20 kernels using a digital Vernier calliper (resolution 0.01 mm) and calculated as:

$$\mathrm{EI} = \frac{\text{Extrudate diameter}}{\text{Die diameter}} \times 100$$

The BD (g/L) was measured as the weight of extrudates corresponding to 1 L. The measurement was realized for a minimum of 5 replicates per treatment.

Before further analysis, the extrudates were dried at 65°C for 48 h. The oil absorption capacity (OAC) was measured on the dried extrudates using the following method. A known amount of kernels (150.0 ± 0.0 g) were hand-coated with excess of fish oil (80.9 ± 10.9 g) using a small-scale vacuum chamber. The chamber was connected to a vacuum pump and pressure was decreased to 200 mbar (over 1 min) while shaking. The pressure was then slowly regained (over 2-3 min) with continuous shaking. The coated pellets were then gently centrifuged (3 min at 48 g) to remove the excess of oil and weighted. The OAC (%) was calculated as follow:

$$\mathrm{OAC} = \frac{\text{Wcoated -Wextrudates}}{\text{Wextrudates}} \times 100$$

Where Wcoated is the weight (g) of the pellets after coating, Wextrudates is the amount (g) of sample used.

Each sample was analysed in duplicates.

Oil leaking (OL) was measured using the weight difference of hand-coated pellets before and after centrifugation. For this purpose 10.0 g of pellets were put in a 50 ml tube with absorbance paper at the bottom. The tubes were centrifuged at 123 g for 5 min and the pellets were weighted. OL (%) was calculated as follow and expressed as the oil loss compare to the sample oil content:

$$\mathrm{OL} = \frac{\text{Initial Weight - Final Weight}}{\text{Sample oil content}} \times 100$$

Where Initial and Final Weight are the weight (g) before and after centrifugation, respectively and the sample oil content is the amount (g) of oil contained in the sample derived from the OAC. Each sample was analysed in duplicates.

The retention in water (RT) was measured as percentage of the initial amount of pellet able to remain dissolved after 24 h in water (internal method, BioMar A/S). Starch gelatinization (SG) was measured on the final pellets using iodine method on cold and hot solution and expressed as a percentage of the total starch content (internal method, BioMar A/S).

Statistical analysis

A total of 26 production batches and 22 variables were used for the statistical analysis (Table 4). The principal component analysis (PCA) was used to explore the dataset and investigate possible relations between variables using FactoMineR package (24). Pearson's correlation test was used in the case of a

TABLE 4
Description of the 22 variables used for the Principal Component Analysis and General Additive Model

Variables	Abbreviation	Type	Use in Principal Component Analysis	Use in General Additive Models (GAMs)
Treatment		qualitative	supplementary qualitative	
Diet		qualitative	supplementary qualitative	Factor, 6 modalities (FM1, FM2, FM3, FeM8, FeM16 and FeM24)
Raw material Fish meal 1	RM.FM1	quantitative	supplementary quantitative	
Raw material Fish meal 2	RM.FM2	quantitative	supplementary quantitative	
Raw material Fish meal 3	RM.FM3	quantitative	supplementary quantitative	
Raw material Feather meal	RM.FeM	quantitative	supplementary quantitative	
Raw material Wheat flour	RM.WF	quantitative	supplementary quantitative	
Water holding capacity	WHC	quantitative	meal mix property	Smooth term
Waper soluble protein	WSP	quantitative	meal mix property	Smooth term
Temperature	T	quantitative	process parameter	Smooth term
Moisture	Moist	quantitative	process parameter	Smooth term
Specific mechanical energy	SME	quantitative	system response	Response
Specific thermal energy	STE	quantitative	system response	Response
Screw back pressure	SBP	quantitative	system response	Response
Expansion index	EI	quantitative	pellet quality indicator	Response
Bulk density	BD	quantitative	pellet quality indicator	Response
Retention time	RT	quantitative	pellet quality indicator	Response
Starch gelatinization	SG	quantitative	pellet quality indicator	Response/Smooth term
Oil absorption capacity	AOC	quantitative	pellet quality indicator	Response
Oil leaking	OL	quantitative	pellet quality indicator	Response
Water holding capacity extrudates	WHC.Ext	quantitative	extrudate property	Response
Waper soluble protein extrudates	WSP.Ext	quantitative	extrudate property	Response

linear relationship between two variables. General additive models (GAMs) were used to describe the non-linear relationship (25). In order to not over fit the data, k=4 was chosen for each descriptive variables. System response and pellet quality indicators variables were tested as response variables of temperature (T) and moisture (Moist), in addition to (i) quantitative variables WSP and WHC or (ii) the qualitative variable Diet (Table 4). The final model for each response variable was selected using the significance of each descriptive variable ($P<0.05$), the description plots (variance of the residuals *vs.* theoretical quantiles, histogram of residual distribution, residuals *vs.* linear prediction, response *vs.* fitted values) and the Akaike criteria. All data analyses were done using R (26).

RESULTS

The results of the extrusion parameters, the meal mix and extrudate properties, the system response and the pellet quality indicators are presented in Table 3. Several levels of the initial design could not be produced for some recipes and led to an unequal number of batches per diet (3 to 6). This is to be remembered for data interpretation as FeM diets were more represented than FM diets.

Data overview

The PCA plots in Figure 1 give an overview of the results and highlight interesting relationships for further study. The first two principal components (PCs) explained 26.3% and 23.1% of the variance (Figure 1A). The projections of the variables show that FeM and WF have low WSP and WHC, opposite of FM1, and that high WSP and WHC of the raw material relate to a high expansion index (EI) and retention in water (RT), and to a low specific thermal energy (STE) and oil leaking (OL). The screw back pressure (SBP) and starch gelatinization (SG) seem to relate closely to the process parameters (T and Moist). The projections on dimension 3 and 4 explain 16.0 and 8.5% of the variance, respectively and show that FM2 was characterized by a high WHC. The oil absorption capacity (AOC) did not seem to relate to other variables (Figure 1A).

The second plot is a mapping of the batch by Diet (Figure 1B). The pellets produced from diets FM1 and FM2 differ significantly in their properties and the effect on system response (not overlapping). The diets formulated with the three different fish meals differ significantly from the diets containing increasing content of feather meal (FeM8, FeM16 and FeM24). Increasing the amount of FeM in the recipe shifted the pellet quality towards lower WSP and expansion index (EI), and higher specific thermal energy (STE) and oil leaking (OL) as seen by comparing Figure 1A and B.

GAM on system response

The results from the GAMs showed that few variables explained the majority of the variance for the specific mechanical energy, specific thermal energy and the screw back pressure. The variance of the specific mechanical energy was described at 83.8% by the variations in WSP and the interaction between T and Moist (Table 5). The WSP and specific mechanical energy had a linear, positive relation (Figure 2A). The highest specific mechanical energy was achieved at the low moisture level, visualized by the yellow area in Figure 2B. The specific thermal energy response was mainly explained by WSP variations (87.5%, data not shown), being the highest for the low WSP

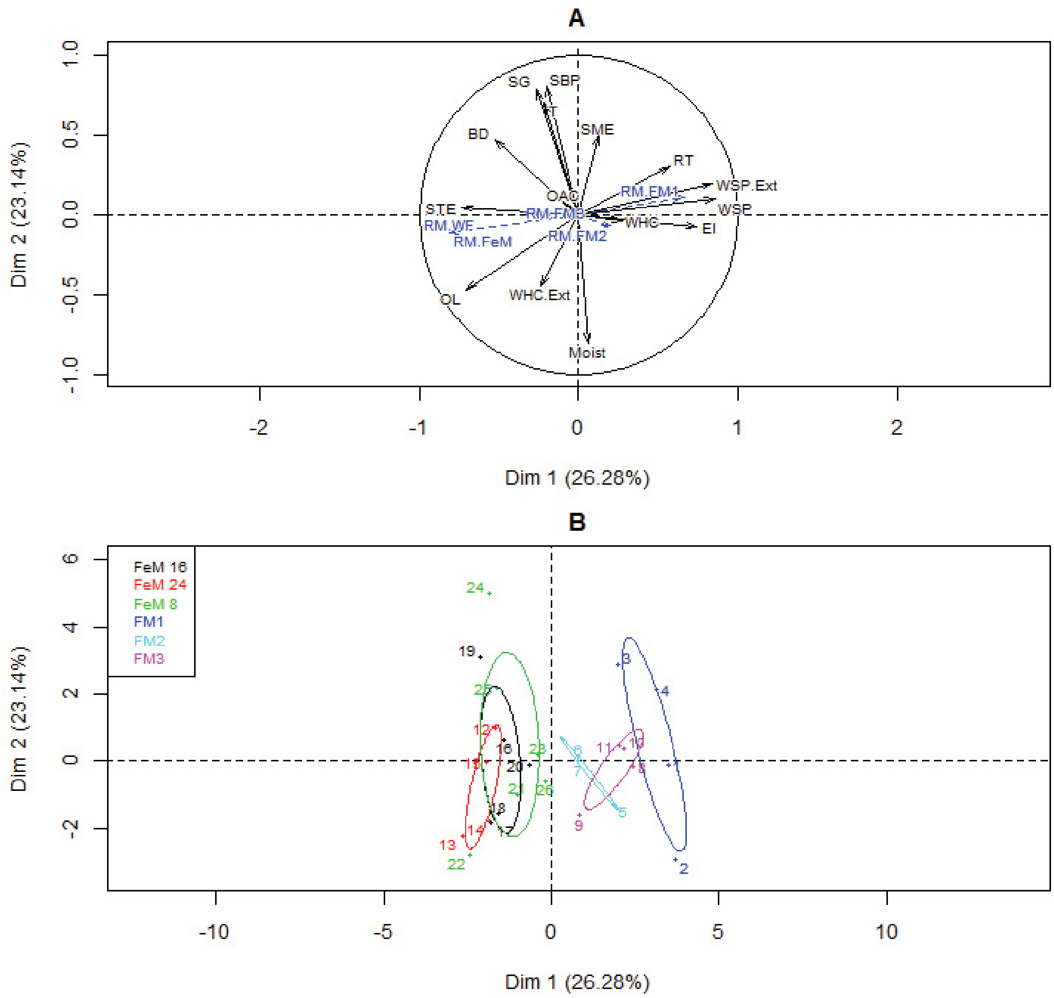

Figure 1A) *Results of the principal component analysis for the 22 variables projected in dimension 1 and 2*

Figure 1B) *Results of the principal component analysis for the 26 production batch projected in dimensions 1 and 2, using confidence ellipse for the variable Diet*

RM.FM1: Raw materials fish meal 1; **RM.FM2**: raw material fish meal 2; **RM.FM3**: raw material fish meal 3; **RM.FeM**: raw material feather meal; **RM.WF**: raw material wheat flour; **WHC**: water holding capacity; **WSP**: water soluble fraction; **T**: temperature; Moist: moisture; **SME**: specific mechanical energy; **STE**: specific thermal energy; **SBP**: screw back pressure; **EI**: expansion index; **BD**: bulk density; **RT**: retention; SG: starch gelatinization; **OAC**: oil absorption capacity; **OL**: oil leaking; **WSP**. Ext: water soluble fraction in extrudate; **WHC**. Ext: water holding capacity of extrudate.

TABLE 5
Results of the general additive models (GAMs) for the system response, pellet quality indicators and extrudates properties

Response variables	Abbreviation	Variable type	Descriptive variables						Interactions				Explained variance	
			WSP	WHC	T	Moist	SG	T*Moist	WSP* Moist	WSP*T	WHC* Moist	WHC*T	Diet (factor)	
Specific mechanical energy	SME	system response	<0.001					<0.001					83.8%	79.3%
Specific thermal energy	STE	system response	<0.001								<0.001		95.4%	93.1%
Screw back pressure	SBP	system response			<0.01	<0.01							79.4%	89.4%
Expansion index	EI	pellet quality indicator	0.02	0.036				0.218					76.0%	76.4%
Bulk density	BD	pellet quality indicator							<0.001				88.4%	31.3%
Retention time	RT	pellet quality indicator									<0.01		92.5%	81.4%
Starch gelatinization	SG	pellet quality indicator			<0.01						<0.01		75.7%	74.1%
Oil absorption capacity	OAC	pellet quality indicator								0.014			65.1%	44.4%
Oil leaking	OL	pellet quality indicator	<0.01	<0.01		<0.01							88.8%	89.2%
Waper soluble protein extrudates	WSP.Ext	extrudate property	<0.001		0.016								94.3%	95.2%
Water holding capacity extrudates	WHC.Ext	extrudate property	0.014				<0.001					0.095	71.6%	60.6%

Only relevant p-values are listed in the table; WSP: water soluble protein; WHC: water holding capacity; T: temperature; Moist: moisture

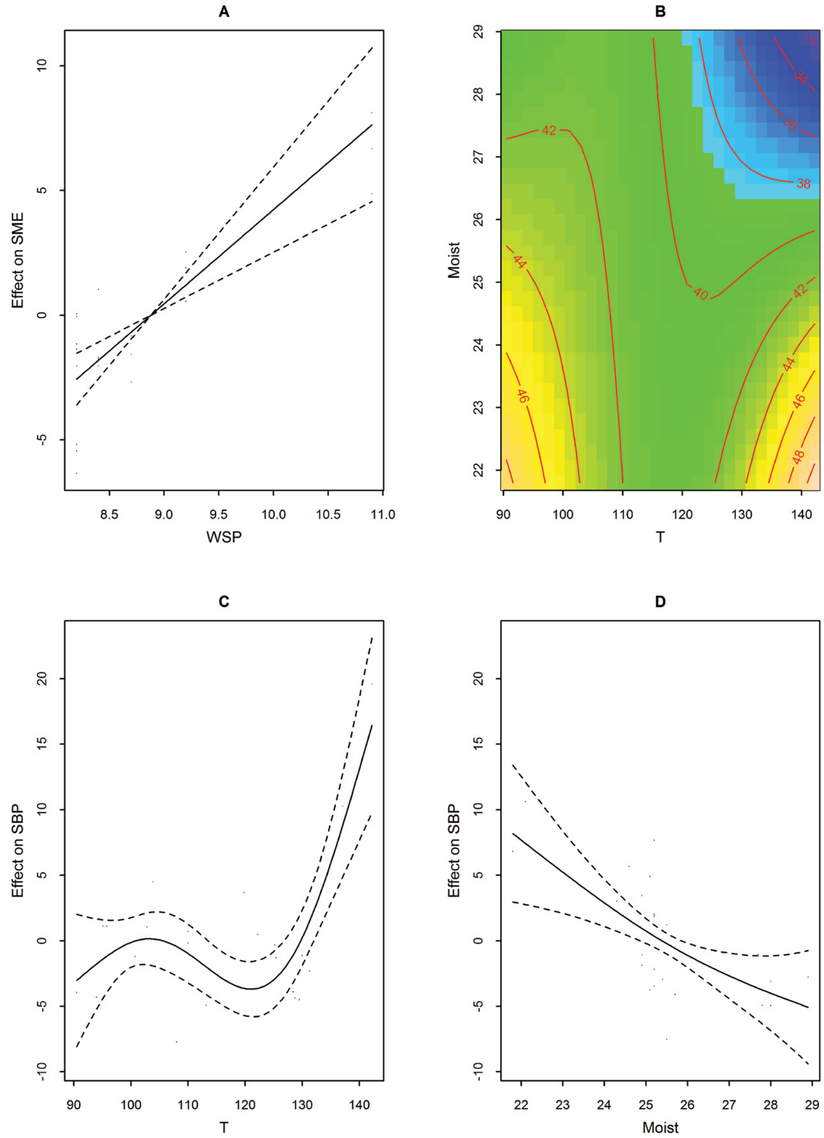

Figure 2A) *Results of the general additive model (GAM) for the system parameters. Effect of the water soluble protein (WSP, %)*

Figure 2B) *Results of the general additive model (GAM) for the system parameters. Interactive effect of temperature (T, °C) and moisture (Moist, %) on the specific mechanical energy (SME).*

Figure 2C) *Results of the general additive model (GAM) for the system parameters. Effect of temperature (T, °C)*

Figure 2D) *Results of the general additive model (GAM) for the system parameters. Moisture (Moist, %) on the screw back pressure (SBP)*

The dotted lines represent the 95 % error limits. The contour lines represent different level of the response variable.

(data not shown), in contrast to the specific mechanical energy response to WSP. The Moist variable had only a marginal effect on specific thermal energy, though significant in interaction with WHC (Table 5), while T had no significant effect. The screw back pressure was mainly explained (81.5% of the variance) by the extrusion parameters T and Moist (Figure 2C and D, respectively). As seen the work required for the extruder engine was minimized at low T and high Moist.

GAM on pellet quality indicators

The GAM analysis show that, few variables can explain the majority of the variance of the pellet quality variables (between 65.1 and 92.5%; Table 5). The expansion index (EI) was mainly affected by meal mix properties (WSP and WHC) and maximal at high WSP and high WHC (Figure 3A and B, respectively). The process parameters (T and Moist) did not have a significant effect on the expansion index (Table 5) but were included in the GAM analysis due to significant loss of variance if removed (54.9 *vs.* 76.0%). The interaction between WSP and Moist alone explained 88.4% of the variance of the bulk density (BD; Table 5). The Moist seemed to have a critical effect for WSP levels above 10% (Figure 3C). The variance of retention in water (RT) was largely explained by Moist during the process in interaction with the WHC (Table 5). Moist had a strong effect on retention values at low WHC (Figure 3D). The degree of starch gelatinization (SG) increased linearly with increasing T (Figure 4A), and was significantly affected by the interaction between WHC and Moist

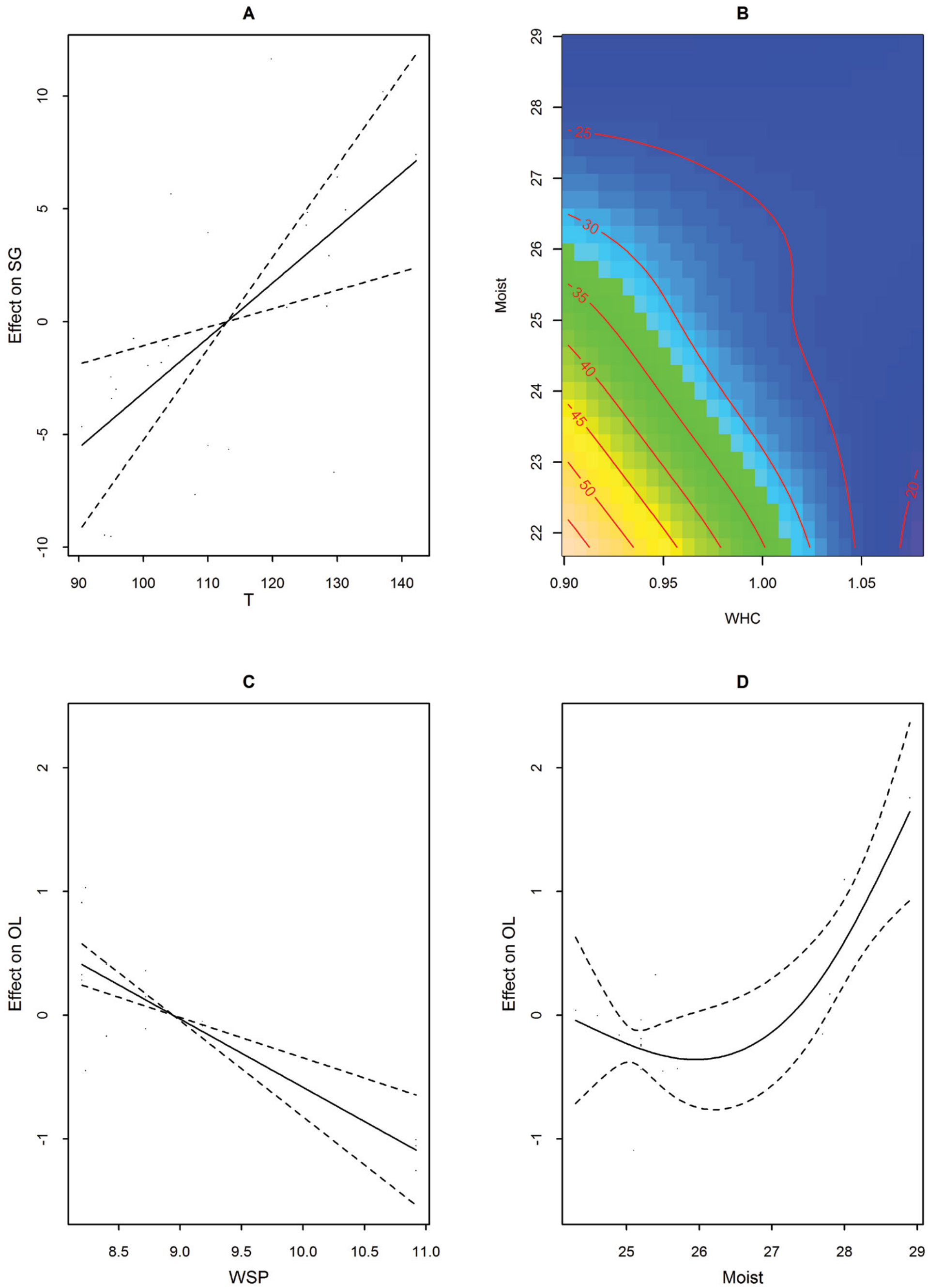

Figure 3A) *Results of the general additive model (GAM) for pellet quality indicators. Effect of water soluble protein (WSP, %)*

Figure 3B) *Results of the general additive model (GAM) for pellet quality indicators.water holding capacity (WHC, g/g) on expansion index (EI)*

Figure 3C) *Results of the general additive model (GAM) for pellet quality indicators. Effect of the interaction between water soluble protein (WSP, %) and moisture (Moist, %) on bulk density (BD)*

Figure 3D) *Results of the general additive model (GAM) for pellet quality indicators. Effect of water holding capacity (WHC, g/g) on retention time in the water (RT)*

The dotted lines represent the 95 % error limits. The contour lines represent different level of the response variable.

(Table 5). The higher degrees of starch gelatinization were achieved at low WHC and low Moist level (Figure 4B). The variations of oil absorption capacity (OAC) were significantly explained by the interaction between the variables WSP and T. The larger effects on oil leaking (OL) were given by WSP and Moist, which on their own explained 54.9 and 46.4% of the variance, respectively (Figure 4C and D, respectively). The WHC had a smaller, but still significant effect and was therefore kept in the model which explained 88.8% of the OL variance (Table 5).

Extrudates properties

The WSP and WHC were measured in the extrudates sampled at the exit of the extruder die. In general, extrusion resulted in a decreased WSP yet the effect differs according to the initial WSP (meal mix) and temperature conditions during extrusion (Table 5). The WHC of the extrudates was consistently higher than of the meal mixes. Using GAM we could not find any significant effect of the WHC of meal mixes, T or Moist alone. However, the WSP of the meal mixes and starch gelatinization had significant effects ($P=0.014$ and $P<0.001$, respectively) on the WHC of extrudates.

GAM with diet as a factor

By using WSP and WHC in the GAMs, we intended to show that protein properties are important variables influencing the extrusion process and the pellet quality. However, meal mixes are complex matrixes, including other nutrient than protein having different properties (e.g. oil as a lubricant or starch as a binder). In a second equation, Diet was used as a factor to investigate the other properties of the meal mixes not accounted for by WSP and WHC.

The explained variance for the system response and pellet quality indicators were differently affected by replacing WSP and WHC by the Diet as a factor (Table 5). The explained variance for screw back pressure was improved by 10% with Diet as a factor. Notably, the explained variance for retention in water, oil absorption capacity and

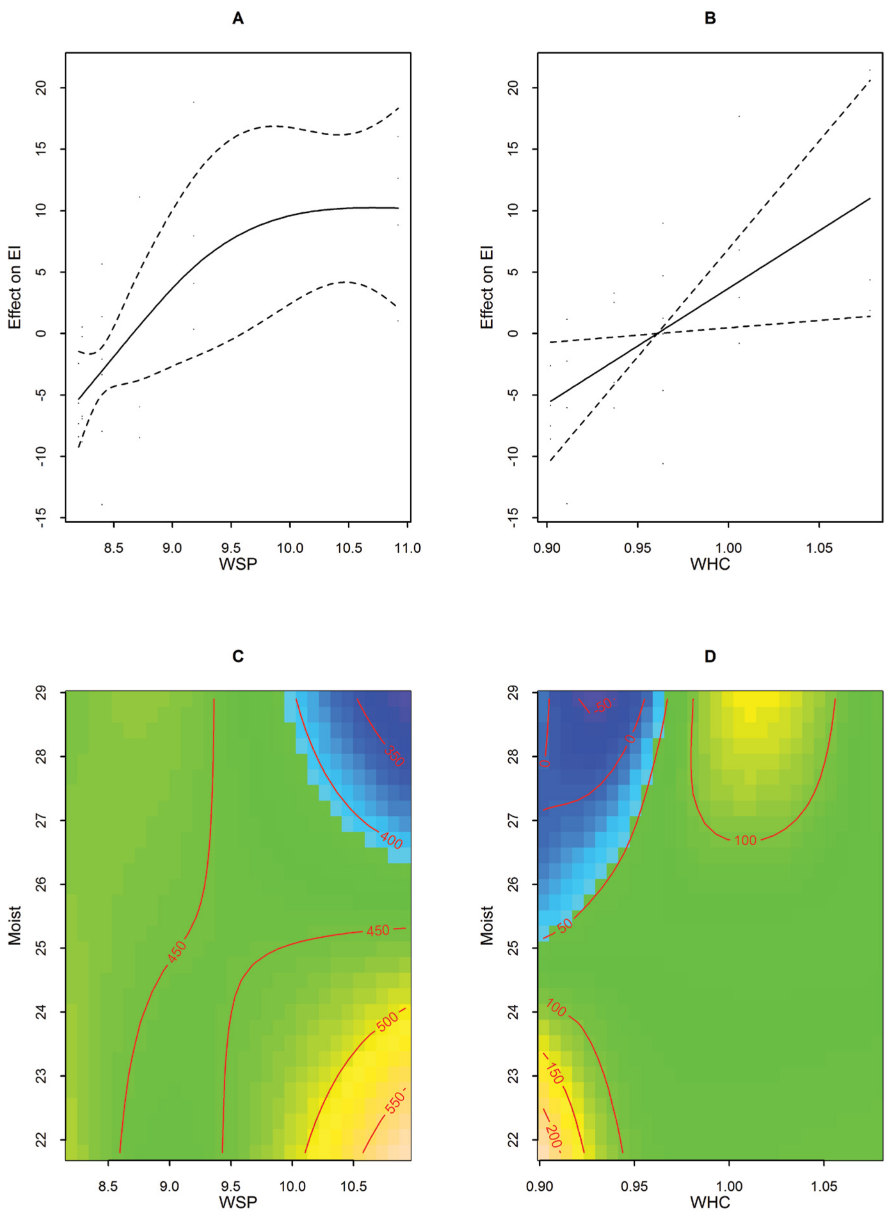

Figure 4A) *Results of the General Additive Model (GAM) for pellet quality indicators. Effect of temperature (T,°C)*

Figure 4B) *Results of the General Additive Model (GAM) for pellet quality indicators. The interaction between water holding capacity (WHC, g/g) and moisture (Moist, %) on starch gelatinization (SG)*

Figure 4C) *Results of the General Additive Model (GAM) for pellet quality indicators. Effect of water soluble protein (WSP, %)*

Figure 4D) *Results of the General Additive Model (GAM) for pellet quality indicators. Moisture (Moist, %) on oil leaking (OL)*

The dotted lines represent the 95 % error limits. The contour lines represent different level of the response variable.

the WHC of the extrudates (WHC. Ext) were significantly higher for the GAM using WSP and WHC (11.1, 20.7 and 11.0%, respectively). The use of Diet as a factor led to a decrease of 57.1% explained variance for bulk density likely due to the fact that the interaction with Moist could not be used.

DISCUSSION

Aquafeeds formulation involves a large number of raw materials with frequent changes in the recipe from one production to another depending on the targeted specie and market, the raw material prices or the available stocks in the warehouse. These changes result in process adjustment to achieve the desired pellet quality, which can vary in unpredictable and undesirable ways. Knowing the raw material characteristics and behaviour through the extruder will aid to predict product properties already at the formulation step and ease the adjustment of process parameters to achieve the desired pellet quality.

Effect of meal mix properties, temperature and moisture level on system response

Overall, increasing WSP led to increased specific mechanical energy, decreased specific thermal energy and had no significant effect on screw back pressure. The WSP fraction of a raw material acts as plasticizer on the dough and has been shown to affect positively the specific mechanical energy (16,27). Similarly to the results obtained in the later study, the WSP in the diets formulated with different fish meal quality and with increasing level of feather meal had a positive effect on the specific mechanical energy. The specific mechanical energy is the energy caused by friction between the shearing device and dough, and WSP seems to increase the resistance of the dough to the shear forces. The range of WSP in the present study (8.23 to 10.92%) was lower than the range reported in a previous study (7.1 to 26% (16). Even though the raw material used in the two studies differed (fish meal quality and feather meal), the positive effect of WSP on the specific mechanical energy was significant in both cases. The small soluble compounds likely increased the frictions between macromolecules, leading to higher specific mechanical energy transfer from the shearing device to the dough (18). The present trial suggests that the soluble protein fraction from feather meal and fish meal might have similar effect on the plasticization of the dough.

The negative effect of WSP on specific thermal energy is likely an indirect effect. Indeed, by increasing WSP in the meal mix, the specific mechanical energy increased and consequently thermal energy input was decreased to regulate temperature (decreased water temperature and barrel heating).

Increasing moisture level from 22 to 28% led to increased specific thermal energy and decreased specific mechanical energy and screw back pressure. As expected, water addition had a major effect on the specific thermal energy because of the hot water contributing to warming up the dough. In addition, as moisture in the dough increases, viscosity decreases, resulting in lower shear forces and specific mechanical energy (28,29). In order to compensate for the loss of temperature originating from the mechanical work, more thermal energy had to be added by heating the extruder barrels. The negative effect of increased moisture level on specific mechanical energy has previously been reported during extrusion of plant based aquafeeds (15). The opposite effect of water and WSP on the energy transfer from the shear force to the dough supports the fact that these plasticizers interfere differently with the raw material matrix (16).

The effect of temperature changes on specific mechanical energy was closely linked to the moisture level as previously reported in extrusion of soybean proteins (29). During extrusion the specific mechanical energy is closely related to the viscosity of the dough and therefore the temperature (30,31). The specific mechanical energy was highest at condition where the dough viscosity would be expected to be high (low temperature, low moisture) and decreased at higher temperature and moisture level. Interestingly, low moisture content (below 25%), and high temperature (above 130°C) tended to increase specific mechanical energy as shown before (28). In the present trial this could be linked to (i) the number of die holes changed for some diets, affecting the shear applied in the final part of the extruder or (ii) changes in protein properties under insufficient plasticization and high temperature.

The variation of screw back pressure was explained solely by the changes in temperature and moisture. The screw back pressure is related to the pressure applied at the die from the dough on the screw when the dough is forced through the die holes. The higher the viscosity, the higher the screw back pressure because more friction are created at the in the die channel resulting in back pressure (32). Temperature and moisture are important parameters affecting the dough viscosity and were the only parameters having a significant effect on screw back pressure in the present trial. As expected, increasing moisture led to decrease screw back pressure, indicating lower viscosity (32,33). However, increasing temperature should decrease viscosity and should result in lower screw back pressure. In the present trial, increasing temperature over 125°C led to increase screw back pressure. This is likely due to the predominant effect of moisture and to the fact that the number of holes on the die plate was increased from 4 to 8 for several diets processed at low temperature to allow for producing pellets. Die plate design had to be adapted to allow the extrusion at low temperature because of the high viscosity built up unwanted high pressure at the die.

Effect of meal mix properties, temperature and moisture levels on pellet quality indicators

Overall the WSP and the WHC had a significant effect on pellet quality. The oil absorption capacity had the lowest explained variance (65.1%) when using GAM. In addition, the oil absorption capacity values did not correlate to density (P=0.69) as previously shown (14,16), which can be explained by the minor differences measured in both bulk density and oil absorption capacity of the extrudates in this experiment (456 ± 29 g/L and 32.8 ± 2.7%, respectively).

The expansion index was the highest for the extrudates produced with the meal mixes having high WSP and WHC, i.e. meal mixes based on fish meals. In the extrusion process, the main forces affecting expansion are steam pressure and dough viscosity. The expansion is driven by high temperature differences between the extruder and the ambient air which will allow for steam to flash off. The viscosity is a resisting force to expansion, meaning the thicker the dough, the lower the expansion. In the present trial, the viscosity was related to the moisture content. Therefore, significant effects of temperature and moisture on expansion index were expected but not detected by the GAM. In order to produce sinking pellet for some of the batches further used in a fish trial, steam was released at the degasser to control the density. This likely led to underestimate effect of temperature and moisture on the expansion. However, by removing the interaction between temperature and moisture in the model, the explained variance decreased significantly (76% to 54.9%). The term was then kept in the model and supports the important of the process parameters on expansion index.

The density is driven by the steam that flashes off at the die and was significantly affected by the WSP in interaction with the moisture level. The optimal values for density at the extruder die (450 g/L, internal knowledge) were obtained with low moisture level and low WSP, which is in agreement with the results from (16). Increasing moisture level had a negative effect on starch gelatinization while it could be expected that increasing the moisture level would improve starch gelatinization as the reaction is related to the available water in the melt (1). The lower viscosity induced by increasing Moist led to decreased specific mechanical energy (34). This likely prevented the starch from receiving enough energy to gelatinize. Recently it was found that adding up to 40% water to the extrusion of aquafeeds based on fish meal and soybean meal improved the expansion, the density and the water stability (35). This finding is in contrast to our results, and can be explained by the high WHC of soybean, which requires higher water addition during extrusion compared to the feather meal or the fish meals used in this study (14). Starch gelatinization might be dependent on the interaction between moisture content and WHC of the protein fraction. At low moisture level, the starch much competes against the protein hydration for the available water (8).

In the present trial, the greatest starch gelatinization was achieved with the meal mixes having a low WHC, which supports that the starch ability to bind water is lower than that of proteins. However, decrease in gelatinization with increasing the moisture in the extruder shows that in modern aquafeed, the starch gelatinization is not limited by the moisture available in the extruder but rather by mechanical energy input. Increasing the temperature had a significant positive effect on the degree of starch gelatinization, which confirms that the reaction is favoured by elevated temperature (1). Temperature changes had limited effect on the other pellet quality parameters in the present setup.

The retention in water was improved and the oil leakage was lowest for the extrudates produced with the meal mixes having high WSP and WHC, i.e. meal mixes based on fish meals. This suggests that the protein structures created with fish meal or feather meal have different properties, likely link to their different ability to texturize. Increasing moisture levels had a negative effect on retention in water and oil leaking likely linked to the decreased viscosity and therefore decreased in texturizing of proteins at the die.

Process effect on extrudates physicochemical properties

Have previously shown that WSP of fish meal was not affected by the extrusion process when using thermal treatment to extract the soluble fraction (16). Therefore the authors concluded that small peptides and amino acid forming the WSP fraction present in the meal were contributing to structural network created during extrusion process. The present results showed that the extrusion process led to a decreased amount of soluble proteins. The discrepancy of the results between the two studies is likely linked to the extraction method. Indeed, extracted proteins using a boiling water-bath which disrupts weak interactions, while the present investigation used water at room temperature where such interactions remain intact (16). Thus, the results from both studies suggest that small peptides and amino acid of the WSP fraction entrapped into the structural network during extrusion process are hold by weak interactions. In addition, the WSP in the extrudates were affected by the temperature supporting the fact that the protein network will change in different process conditions.

The increased WHC after extrusion could be linked to the properties of the protein network as suggested by the effect of the WSP fraction in the GAM analysis. However, the strongest effect in the GAM was given by the degree of starch gelatinization, likely due the higher ability of starch to entrap water in the gelatinized state compare to the native state (36).

Using WSP and WHC to describe the raw material effect on extrusion system response and pellet quality

Meal mixes are complex matrixes involving a large number of compounds (starch, proteins, fibres, fat, solubles) with different properties and susceptible to affect the process and the quality of the final product. Work has been done on characterizing the protein raw materials and often WSP and WHC appeared to be important criteria (15,16,32).

The use of hydrolysed feather meal and fish meal in this study resulted in similar output compared to studies based on different fish meal qualities (8,16,21). Fish meal and feather meal differ significantly in their protein properties. Feather meal is mainly made of keratins which are structural proteins strongly held together by disulphide bridges and containing hydrophobic groups. Fish meal is mainly composed of myofibrillar proteins (37). In order to be digested, feather must undergo an extensive process (hydrolysis, cooking) in order to denaturize the strong structure and as a result, feather meal has little or no protein remaining in the native state and low WSP. The quality of fish meal is linked to the raw material type and process applied. Mild cooking and reinjection of the stick water will result in higher quality with higher WSP (38).

Replacing WSP and WHC in the GAM by the factor Diet did not improve the explained variance for the specific mechanical energy and the specific thermal energy suggesting that these two parameters were able to model the meal mix effect. However, the screw back pressure was improved by using Diet as a factor, suggesting that other parameters, which are not accounted for in the WSP and the WHC are of importance for optimizing this variable. The oil amount in the meal mix will be a major contributor to the viscosity of the dough (lubricant effect) and thus susceptible to affect differently the extrusion process and the final pellet quality. Similarly, the starch level will affect the dough properties and differed slightly between the recipes. The meal mixes were grinded and sieved through a 0.75 mm mesh but difference in particle size could have subsisted and affected the homogeneity of the mash.

Characterization of the meal mix by the WSP and the WHC in the GAM analysis explained well the effect on the pellet quality. The differences between the models with WSP and WHC or with Diet as a factor can be linked to changes in the wheat flour level (from 18 to 24 g/100 g), which has strong effect on pellet quality (6). Pellet quality will also be strongly affected by the ability of proteins to build a strong network during extrusion. This is related to the cross-linking between amino acids occurring in the final section of the extruder, just before the die mainly though di-sulphide bounds (39). Fish meal and feather meal have different amino acid composition (especially sulphur containing amino acid) and therefore will have different ability to texturize and form a protein network.

Characterization of raw material functional properties and behaviour during process could be a major advantage to adjust the extrusion parameters and achieve the desired pellet quality. The results suggest that no matter the protein source the two indicators (WSP and WHC) could describe the raw material effect on extruder response during process and on pellet quality to some extent. Further research using other raw materials (plants, animal meal) should be conducted. In addition, the effect of other raw material properties should be studied to describe better some variables which had a low explained variance (oil content, starch content, amino acid profile).

CONCLUSION

The GAM analysis suggested that few variables could explain changes in the system response (measured as specific mechanical energy, specific thermal energy and screw back pressure) and pellet quality indicators (expansion index, bulk density, retention in the water, starch gelatinization, oil absorption capacity and oil leaking). In general, the WSP had a significant effect on the specific mechanical energy and specific thermal energy, as well as on the expansion index and oil leaking. The screw back pressure, linked to the dough viscosity was affected only by temperature and moisture changes. The WHC had less effect than WSP on the system response and pellet quality indicators, likely due to the low WHC value of the raw materials (fish meal and feather meal). Replacing WSP and WHC by Diet as a factor in the GMAs improved the explained variance for screw back pressure and decreased the explained variance for bulk density and oil absorption capacity. Yet, in the present setup WSP and WHC could describe the raw material effect. The current model is based on six recipes formulated with four different protein raw materials. Surely more research and modelling covering a wider range of protein sources and raw material parameters is needed to support the results.

ACKNOWLEDGEMENT

The authors would like to thanks the operating team at BioMar A/S Tech Center, Brande, Denmark for their help and patience during the trial. The authors would also like to thanks Thomas Cavrois for conducting analysis on the pellet quality. The present study is part of a research project granted by the Innovation Fund Denmark (grant number 69-2014-1) and was co-financed by BioMar A/S, Copenhagen University, Aarhus University and the Technical University of Denmark.

REFERENCES

1. Sorensen M. A review of the effects of ingredient composition and processing conditions on the physical qualities of extruded high-energy fish feed as measured by prevailing methods. Aquacult Nutr. 2012;18:233-48.
2. Wolska J. Towards a better understanding and control of factors affecting the physical quality of fish feed. 2014;152.
3. Aarseth KA, Perez V, Boe JK, et al. Relieable pneumatic conveying of fish feed. Aquacult Eng. 2006;35:14-25.
4. Hansen JO, Storebakken T. Effects of dietary cellulose level on pellet quality and nutrient digestibilities in rainbow trout (Oncorhynchus mykiss). Aquaculture. 2007;272:458-65.
5. Sorensen M, Nguyen G, Storebakken T, et al. Starch source, screw configuration and injection of steam into the barrel affect the physical quality of extruded fish feed. Aquacult Res. 2010;41:419-32.
6. Sorensen M, Morken T, Kosanovic M, et al. Pea and wheat starch possess different processing characteristics and affect physical quality and viscosity of extruded feed for Atlantic salmon. Aquacult Nutr. 2011;17:326-36.
7. Kraugerud OF, Jorgensen HY, Svihus B, et al. Physical properties of extruded fish feed with inclusion of different plant (legumes, oilseeds, or cereals) meals. Anim. Feed Sci Technol. 2011;163:244-54.
8. Samuelsen TA, Mjos SA, Oterhals A, et al. Impact of variability in fishmeal physicochemical properties on the extrusion process, starch gelatinization and pellet durability and hardness. Anim Feed Sci Technol. 2013;179:77-4.
9. Nutrient Requirements of Fish and Shrimp. The National Academic Press, Washington, DC.2011.
10. Bendiksen EA, Johnsen CA, Olsen HJ, et al. Sustainable aquafeeds: Progress towards reduced reliance upon marine ingredients in diets for farmed Atlantic salmon (Salmo salar L.). Aquaculture.2011;314:132-39.
11. Borquez A, Serrano E, Dantagnan P, et al. Feeding high inclusion of whole grain white lupin (Lupinus albus) to rainbow trout (Oncorhynchus mykiss): Effects on growth, nutrient digestibility, liver and intestine histology and muscle fatty acid composition. Aquacult Res. 2011;42:1067-78.

12. Burr GS, Wolters WR, Barrows FT, et al. Replacing fishmeal with blends of alternative proteins on growth performance of rainbow trout (Oncorhynchus mykiss), and early or late stage juvenile Atlantic salmon (Salmo salar). Aquaculture. 2012;337:110-16.

13. Collins SA, Desai AR, Mansfield GS, et al. The effect of increasing inclusion rates of soybean, pea and canola meals and their protein concentrates on the growth of rainbow trout: Concepts in diet formulation and experimental design for ingredient evaluation. Aquaculture. 2012;349:90-9.

14. Draganovic V, Van Der Goot AJ, Boom R, et al. Assessment of the effects of fish meal, wheat gluten, soy protein concentrate and feed moisture on extruder system parameters and the technical quality of fish feed. Anim Feed Sci Technol. 2011;165:238-50.

15. Draganovic V, Boom RM, Jonkers J, et al. Lupine and rapeseed protein concentrate in fish feed: A comparative assessment of the techno-functional properties using a shear cell device and an extruder. J Food Eng. 2014;126:178-89.

16. Oterhals A, Samuelsen TA. Plasticization effect of solubles in fishmeal Food Res. 2015;69: 313-21.

17. Zhao W, Yang R, Zhang Y, et al. Sustainable and practical utilization of feather keratin by an innovative physicochemical pretreatment: High density steam flash-explosion. Green Chem. 2012;14:3352-60.

18. Zhang Y, Yang R, Zhao W, et al. Improving digestibility of feather meal by steam flash explosion. J. Agric Food Chem. 2014;62:2745-51.

19. Hartviksen M, Vecino JLG, Ringo E, et al. Alternative dietary protein sources for Atlantic salmon (Salmo salar L.) effect on intestinal microbiota, intestinal and liver histology and growth. Aquacult Nutr. 2014;20:381-98.

20. Idris A, Vijayaraghavan R, Rana UA, et al. Dissolution of feather keratin in ionic liquids. Green Chem. 2013;15:525-34.

21. Samuelsen TA, Mjos SA, Oterhals A, et al. Influence of type of raw material on fishmeal physicochemical properties, the extrusion process, starch gelatinization and physical quality of fish feed. Aquacult Nutr. 2014;20;410-20.

22. Immergut EH, Marck FM. Principles of plasticization. In Plasticization and Plasticizer Processes. 1965;1-26.

23. Keller RP, Neuville MC. Determination of total protein in human milk: Comparison of methods. Clin Chem. 1986;31:120-23.

24. Husson F, Josse J, Mazet J, et al. FactoMineR: multivariate exploratory data analysis and data mining with R.2008.

25. Hastie T, Tibshirani R. Generalized additive models. Stat Sci 1986;3:297-18.

26. A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. 2013.

27. Osen R, Toelstede S, Wild F, et al. High moisture extrusion cooking of pea protein isolates: Raw material characteristics, extruder responses, and texture properties. J Food Eng. 2014;127:67-4.

28. Rosentrater KA, Muthukumarappan K, Kannadhason S, et al. Effects of Ingredients and Extrusion Parameters on Properties of Aquafeeds Containing DDGS and Corn Starch. J Aquacult Feed Sci Nutr. 2009;1:44-60.

29. Chen FL, Wei YM, Zhang B, et al. System parameters and product properties response of soybean protein extruded at wide moisture range. J Food Eng. 2010;96:208-13.

30. Bhattacharya M, Hanna MA. Influence of process and product variables on extrusion energy and pressure requirements. J Food Eng 1987;6:153-63.

31. Akdogan H. Pressure, torque, and energy responses of a twin screw extruder at high moisture contents. Food Res Int. 1996;29:423-29.

32. Osen R, Toelstede S, Wild F, et al. High moisture extrusion cooking of pea protein isolates: Raw material characteristics, extruder responses, and texture properties. J Food Eng. 2014;127:67-4.

33. Hayashi N, Hayakawa I, Fujio Y, et al. Flow behaviour of soy protein isolate melt with low and intermediate moisture levels at an elevated temperature. J Food Eng 1993;18:1-11.

34. Jongsutjarittam O, Charoenrein S. The effect of moisture content on physicochemical properties of extruded waxy and non-waxy rice flour. Carbohydr Polym. 2014;114:133-40.

35. Umar S, Kamarudin MS, RamezaniFard E, et al. Physical properties of extruded aquafeed with a combination of sago and tapioca starches at different moisture contents. Anim Feed Sci. Technol. 2013;183:51-5.

36. Majzoobi M, Radi M, Farahnaky A, et al. Physicochemical properties of pre-gelatinized wheat starch produced by a twin drum drier. J Agric Sci Technol, 2011;13:193-02.

37. Suzuki T. Fish and Krill Protein: Processing Technology. 1981;260.

38. Aksnes A, Mundheim H. The impact of raw material freshness and processing temperature for fish meal on growth, feed efficiency and chemical composition of Atlantic halibut (Hippoglossus hippoglossus). Aquaculture. 1997;149;87-06.

39. Manoi K, Rizvi SSH. Physicochemical changes in whey protein concentrate texturized by reactive supercritical fluid extrusion. J Food Eng. 2009;95:627-35.